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THE TRANSACTIONS OF THE ZOOLOGICAL SOCIETY OF LONDON

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Clerk of Publications . . . G. B. STRATTON, M.B.E.

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SOME EFFECTS OF OESTRADIOL BENZOATE ON THE REPRODUCTIVE DUCTS OF THE FEMALE DOGFISH *SCYLIORHINUS CANICULUS*

BY

J. M. DODD* AND C. K. GODDARD†

Gatty Marine Laboratory, The University, St. Andrews

[Accepted 14th February, 1961]

(With 1 plate)

The effects of oestrogens on the reproductive ducts and copulatory structures of female higher vertebrates are well known : these results are supplemented by the present work in which pellets of oestradiol have been implanted intramuscularly in female dogfish *Scyliorhinus caniculus* at different stages of sexual maturity. This treatment was found to have a pronounced stimulatory effect on the oviducts of certain of the treated fish which was obvious on macroscopic examination. Secretion of horny egg-case material was especially stimulated. Sexually immature specimens were not affected and the oviducts of mature egg-laying fish did not appear to show increased stimulation. The group which responded to the implanted steroid appeared to be about to mature, though vitellogenesis had not yet started in the most stimulated specimen. The cloacal region appeared also to be stimulated in the fish of this particular group. Rate of absorption of the oestradiol from the implant was low, the most stimulated fish having absorbed approximately 3 mg. in seven months. It is suggested that at this low rate of absorption the stimulatory effects are likely to be of a physiological nature. These results acquire additional significance in the light of the recent discovery of oestradiol 17β in the ovaries of *Squalus suckleyi*.

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INTRODUCTION

Recent work of Chieffi (1952, 1954) and Thiebold (1954) has shown that the embryonic gonads and primordial reproductive ducts of elasmobranchs are affected by sex steroids, and there is an extensive literature dealing with the effects of androgens and oestrogens on the gonads, reproductive ducts and secondary

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sexual characters of other cold-blooded vertebrates (for reviews see Pickford & Atz, 1957, and Dodd, 1955, 1960 a, b). Yet little is known concerning these aspects of reproductive physiology in adult elasmobranchs. This gap in our knowledge is the more remarkable since elasmobranchs have well differentiated reproductive ducts, the detailed structure of which has been described in several species (Borcea, 1906 ; Daniel, 1934 ; Matthews, 1950). Moreover, secondary sexual characters are present in both sexes : these take the form of claspers in the male and modifications of the histology and morphology of the cloaca in the female.

It seems likely that some aspects of growth and differentiation of the claspers are under steroid control, though the experimental evidence in support of this is meagre. Hisaw & Abramowitz (1938) treated *Mustelus canis* with " male hormone " and reported that it accelerated growth of the claspers. Goddard & Dodd (in Dodd, 1955) obtained an average length increase of 15 per cent in the claspers of immature *Raia radiata* as a result of injecting large amounts of testosterone propionate. Dodd (1955) makes the point that the target tissues of immature fish may well be relatively insensitive to steroids and suggests that this, and possible hormone specificity, should be taken into account when these results are evaluated. He also suggests that experiments with older fish might yield more striking results since it is usually accepted that the sensitivity of reproductive tissues to their specific hormones rises with the onset of sexual maturity.

The external secondary sexual characters of the female are limited to the cloaca ; it is therefore not surprising that only one investigation is recorded on the effects of oestrogens on the female elasmobranch. In a brief report on work in progress, Hisaw & Abramowitz (1938) note that " female sex hormone " produces a maturation of the genital tract of yearling females of *M. canis* equivalent to that seen in a normal three year old specimen.

In the work reported here we have investigated the effects of oestradiol benzoate on the genital ducts and secondary sexual characters of mature (Pl. 1, fig. 1), immature (Pl. 1, fig. 2), and maturing (Pl. 1, fig. 3) females of the spotted dogfish *Scyliorhinus caniculus*.

MATERIAL AND METHODS

The fish used in these experiments were obtained from the Marine Station, Millport, and brought to St. Andrews by sea and road. During the experimental period they were kept in tanks of 800 gallon capacity in running, aerated sea water, the temperature of which varied over the period of the experiment between 6°C and 15°C. Minced herring was used as food and it was administered once per week by the manual method of force-feeding recently described by Dodd, Dodd & Goddard (1959).

Twelve female fish, varying in reproductive condition from immature to fully mature, and in weight from 410 g. to 1,200 g., were each implanted with a pellet of oestradiol benzoate weighing approximately 25 mg., the exact weight of which was determined. A small incision was made in the epiaxial flank muscles, just above the lateral line, and some 6 cm. behind the last gill pouch ; a trochar was then inserted and gently forced forward through the muscles at

a depth of approximately 0·5 cm. below the skin. A steroid pellet was placed in the barrel of the trochar and lodged in the tissues. In this way deep insertion was achieved with little damage to the tissues, and the muscles acted as a seal. Six fish were implanted in a similar way with a small glass bead. The weights of these sham-implanted fish were selected so that they would act as controls for the entire weight range of the experimental animals. Certain data relevant to the two groups are recorded in Table 1.

Most of the fish were autopsied 7 months (196 days) after implantation, though, as Table 1 shows, five fish in the experimental group and two control fish were sacrificed earlier. Relevant gravimetric data were obtained from all fish at the time of autopsy and a detailed macroscopic examination was made of ovary and oviducts, including contents and hymens when present ; the condition of the cloaca was also recorded. These structures were not examined for microscopic histological changes.

RESULTS

The amount of steroid absorbed by experimental fish which survived 196 days varied between 2·8 mg. and 3·5 mg., i.e. between 11 per cent and 14 per cent of the available dose. The higher figure represents the amount absorbed by the most-stimulated fish.

a. *Immature and maturing fish*

It can be seen by reference to Table 1 that a low value for weight of ovary and oviducts relative to weight of body seems to indicate immaturity, and that, in general, females weighing less than 560 g. are immature. However, there are exceptions which can, we believe, be identified by a careful examination of the ovary, this organ in the immature fish containing only small eggs, whereas an ovary which is small because it is in a resting condition after a period of activity, would be expected to contain corpora lutea and corpora atretica in addition to small oocytes. Fish No. 11, (Pl. 1, fig. 3) is an example of a fish of large size having a small ovary which, on the above criteria, would be classified as immature. The oviducts of this fish showed the greatest degree of stimulation seen. They were extremely hyperaemic and apparently fully differentiated ; they were also tightly packed with the horny tendril-like material by which the egg cases of oviparous elasmobranchs are attached to submarine structures. This condition has never previously been seen in any of the several hundred untreated female dogfish we have examined for other reasons. The degree of stimulation shown by the cloaca of this fish was not commensurate with the highly stimulated oviducts, though the cloaca was mature in appearance. The oviducts were open at autopsy ; unfortunately, their condition in this respect at the start of the experiment was not recorded. Fish No. 4 is an immature specimen of more usual size, though the ovary was in the early growth phase. The stimulatory action of the oestradiol implant on oviducts and cloaca in this fish was marked, though less striking than that just described for the previous specimen. Fish No. 8, though larger than No. 4, is closely similar to it in all important respects. The ovary was growing, though none of the oocytes had a diameter greater than 0·4 cm. At autopsy,

its oviducts were similar to those of a mature fish and contained a great amount of " tendril " secretion ; the cloaca also showed signs of stimulation. Control specimens at a similar stage of development showed no accumulation of horny secretion, and well developed oviducts were found only in fish with fairly mature ovaries.

Immature fish of small size in which the ovary/body weight ratio was less than 0·02 never showed any stimulatory signs after oestradiol treatment and the oviducts remained closed.

b. *Mature fish*

Fish in this category are found both in the experimental and the control group, and treatment with oestradiol failed to produce any effects visible on careful macroscopic examination which were not also present in the untreated specimens. In particular, the excessive amounts of purse secretion characteristic of stimulated fish were not seen. It is possible that a microscopic examination of sections of the oviduct would have yielded signs of stimulation, but this was not carried out.

Subsidiary findings

Three of the treated fish laid eggs at regular intervals throughout the experiments (see Table 1) ; it may therefore be concluded that oestradiol, at least under the conditions obtaining in the present experiment, has no adverse effect on the dogfish ovary, nor does it appear to depress the ovulation-inducing function of the pituitary.

In none of the stimulated fish was secretion of an actual egg case induced, though we have on several previous occasions encountered fully fabricated egg cases in both oviducts in the absence of eggs, so that there is apparently no reason why this degree of stimulation should not have been obtained.

It may also be noted that ciliation of the body cavity (Metten, 1939), a sex-limited character in elasmobranchs, found only in the mature female, was fully developed in the stimulated fish, though it was absent from non-stimulated immature specimens. It seems likely that it was induced by the oestradiol treatment though this cannot be stated with certainty since it is not known whether or not active cilia were present at the time of implantation.

DISCUSSION AND CONCLUSIONS

Most previous workers investigating the effects of steroids on cold-blooded animals have administered them either in an oily solution, or suspended in water. However, among other disadvantages, this technique does not allow unused steroid to be recovered and estimated. The method of implanting pellets of fused steroid should ensure a steady low supply of hormone to the tissues and the amount liberated during the course of the experiments can be easily calculated. Forbes (1941) used this technique with success in lizards (*Sceloporus floridanus*), in which oestrone pellets were implanted ; steroid was absorbed from these pellets at an average rate of approximately 1·6 mg. per six and a half weeks. This rate is higher than that observed in the present work in which 3·2 mg. were absorbed by the most-stimulated fish in seven months.

Table 1 : Fish 1—12, Effects of implanting oestradiol benzoate in female specimens of *S. caniculus* at various stages of sexual maturity. Fish 1C—6C, similar data for control-implanted fish.

No. of fish	Weight (g.)	Length (cm.)	Wt. ovary / Wt. body	Wt. oviducts / Wt. body	Survival time (days)	Findings at autopsy	
						Condition of ovary	Condition of oviducts
1	345	48·0	0·010	0·008	64	immature	immature ; closed
2	402	50·0	0·012	0·006	98	immature	immature ; closed
3	435	52·8	0·018	0·012	196	immature	immature ; closed
4	550	58·3	0·022	0·034	196	immature	highly stimulated ; open
5	559	56·0	0·030	0·027	196	immature but some growing oocytes	intermediate in size ; closed
6	710	60·5	0·024	0·025	21	immature but some growing oocytes	immature ; closed
7	755	59·0	0·040	0·031	196	mature	normal for mature fish; open
8	757	60·0	0·020	0·029	196	immature but some growing oocytes	moderately stimulated ; open
9	896	60·8	0·046	0·037	141	mature	normal for mature fish ; eggs laid and in oviducts
10	902	60·3	0·038	0·030	196	mature	normal for mature fish ; eggs laid and in oviducts
11	937	62·5	0·012	0·032	196	immature	highly stimulated ; open (Pl. 1, Fig. 3)
12	1,172	68·8	0·052	0·035	84	mature	normal for mature fish ; eggs laid
1C	334	48·0	0·011	0·007	64	immature	immature ; closed
2C	362	53·0	0·012	0·006	196	immature	immature ; closed
3C	497	53·8	0·015	0·010	72	Small ovary, but oocytes in early growth phase	immature ; closed
4C	615	59·0	0·028	0·027	196	intermediate ovary	intermediate in size ; open
5C	773	62·0	0·060	0·034	196	mature	normal for mature fish ; open
6C	1,030	65·6	0·048	0·036	196	mature	normal for mature fish ; eggs in oviducts

Any effects resulting from such a low rate of absorption are probably of a physiological nature.

Work on mammals and certain lower vertebrates has shown that oestrogens stimulate the genital ducts and copulatory structures of the female sex, and the results reported above are evidence for the view that they have a similar effect in female dogfish. Further, the cloaca, which is a narrow, dry, poorly vascularised cleft in the immature female elasmobranch, becomes a wide, hyperaemic, mucoid, easily distensible cavity at maturity and we have obtained some evidence that these transformations also are under steroid control.

It seems reasonable to conclude from the above experiments, though the number of positives is small, that the steroid implants had a stimulatory effect on secretory activity of the lower region of the oviducal gland and that this resulted in the production of large amounts of horny " tendril " material which distended the oviducts of the most-stimulated fish throughout their length. Furthermore, there appears to have been a general histological stimulation of the oviducts of certain of the implanted fish involving all regions ; only in this way can the fact be explained that these fish have the ovary/body weight ratio of immature fish whilst the oviduct/body weight ratio is that of mature or maturing specimens. These stimulated fish are believed to be approaching the advent of sexual maturity and it may well be that their reproductive tissues do not become sensitive to steroids until this time : this view is widely held for other vertebrates, in which there is good evidence for it.

The fact that juvenile specimens of *S. caniculus* are unresponsive to the implanted steroid is in contrast to the results of Hisaw & Abramowitz (1938) reported above. These workers found that the immature oviducts of yearling females of the smooth dogfish (*M. canis*) were so stimulated by female sex hormone that they resembled the condition found at sexual maturity. In the case of *S. caniculus*, we take our negative results to indicate that the target tissues in immature specimens are insensitive to steroid influence, at least at the level circulating in these experiments. However, the possibility cannot be entirely excluded that a higher absorption rate over a longer period might have produced a response.

The fact that mature egg-laying fish did not appear to be additionally stimulated by the implanted oestrogen is capable of a number of different explanations. It may be that further stimulation is impossible at any circulating level of hormone, or we can assume that the implant is incapable of producing a sufficiently high titre of hormone to stimulate the mature oviducts, even though it was high enough to produce a marked effect in the maturing female. The other, perhaps more likely, explanation is that some stimulation does, in fact, occur which cannot be recognised macroscopically in the already large and active oviducts of the mature fish. It is certain that stimulation of the secretory processes would be difficult to recognise in an egg-laying female in which the oviducal glands are already extremely active, and in which purse secretion would not accumulate but would be shed with the eggs. The evidence adduced above does not enable us to choose between these views, though we believe that it does enable us to say that oestradiol is capable of inducing hypertrophy of the oviducts, stimulation of secretion by the oviducal gland,

and maturation of the cloaca in maturing dogfish. These findings assume added interest in the light of the recent discovery of Wotiz, Boticelli, Hisaw & Ringler (1958), that the ovaries of *Squalus suckleyi*, in fact, contain oestradiol 17β.

ACKNOWLEDGMENTS

This work was supported by generous grants from the Nuffield Foundation, and from the Browne Fund of the Royal Society. We should like to express our gratitude also to Dr W. Tindall and Dr D. B. Dekanski of Messrs Organon for donating the oestradiol implants, and to the Director and Staff of the Biological Station, Millport, Scotland, for their kindness in providing the experimental fish.

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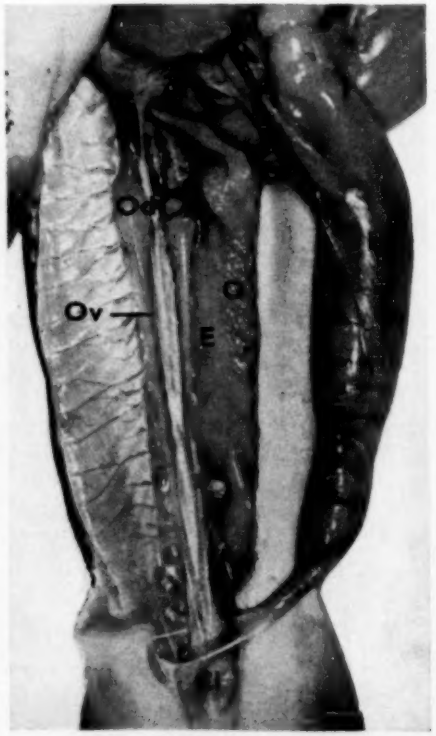
EXPLANATION OF PLATE

Plate 1

Scyliorhinus caniculus : Body cavities opened to display ovaries and oviducts. 1. Untreated mature female ; note ovary with large eggs and right oviduct opened to display egg in horny case. 2. Immature female control ; note small ovary and immature oviducts closed at their junctions with the cloaca. 3. Specimen with immature ovary seven months after implantation of oestradiol benzoate ; note greatly stimulated oviducts, right oviduct distended with horny secretion, left oviduct opened to show tightly packed horny material. (gut removed).

Cl. cloaca ; E. epigonal organ ; I. intestine ; L. liver ; M. right oviduct opened to show egg in horny shell (mermaid's purse) ; O. ovary ; Od. oviducal gland ; Od^1. left oviducal gland opened ; Ov. oviduct ; Ov^1. left oviduct opened, note tightly packed " tendril " material.

1



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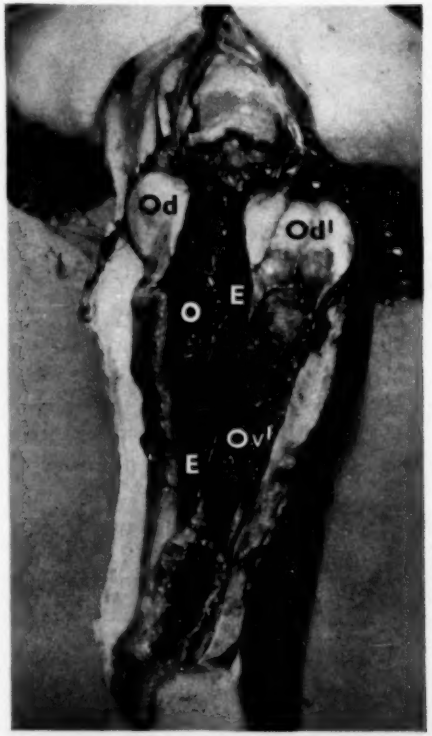

3

Scyliorhinus caniculus. Body cavities opened to display ovaries and oviducts

ON THE CRANIAL MORPHOLOGY OF ICHTHYOSAURS

BY

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Department of Geology, University College of South Wales and Monmouthshire

[Accepted 14th February, 1961]

(With 18 figures in the text)

One of the difficulties in the study of ichthyosaurs has been the lack of ossification, said to be a common characteristic of aquatic groups. This difficulty has been partially removed by the discovery of a specimen of *Ophthalmosaurus* which is particularly well ossified, revealing clear facets which are not usually present. This specimen, together with well preserved skulls of *Ichthyosaurus*, *Leptopterygius* and *Stenopterygius* and many other isolated elements and fragments of *Ichthyosaurus* s.l. formed a basis for the interpretation of the ichthyosaur skull which, with literature of Triassic forms, has led to the recognition of a large number of ancestral ichthyosaurian features. The principal theories of the origin of ichthyosaurs have been examined in the light of this new evidence and shown to be untenable. In particular, von Huene's and Nielsen's theories of direct descent from amphibia are criticised and it is shown that Romer's suggestions of pelycosaur affinities of the ichthyosaurs are mistaken. The relationships of ichthyosaurs are then discussed in terms of the fuller knowledge of ichthyosaur anatomy and of recent literature on primitive reptiles and amphibia. Ichthyosaurs are shown to have common ancestry with chelonians and the ichthyosaur-chelonian stock is considered to be derived from a more primitive stock evolving towards procolophonids or towards procolophonids and pareiasaurs. It is shown that otic conditions superficially similar to those of captorhinomorphs and pelycosaurs but which are, in fact, " sauropsid " may occur in several groups, including ichthyosaurs. The evolution of the temporal region is discussed and used to suggest that ichthyosaurs and chelonians had their origins in *Deuterosaurus* (Permian) times or later but probably not later than the close of the Permian.

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HISTORY OF RESEARCH

Ichthyosaurs have been known since 1708 (Baier, 1708) but the first useful description was given by Home in 1814. Since then a copious literature has been written (see references) from which a number of works may be selected as land-marks from the point of view of cranial morphology.

De la Beche & Conybeare (1821) gave the first good account of the skull, but unfortunately the illustrations were composites. Conybeare (1822) extended the descriptions, corrected errors and gave a conjectural diagram of the occipital aspect of the skull. With the exception of the temporal region, the roof and sides of the skull were accurate but, because of insufficiently good preservation, his accounts of the palate and occiput were seriously in error. The first binominal names were allocated in his paper.

Descriptions and differentiations of species continued to be made and Owen (1881) brought together the knowledge of the Liassic forms.

Baur (1887) followed Cope's identifications of the otic bones and correctly identified the supratemporal and, below this, the squamosal. These and many other morphological observations led him to place ichthyosaurs with *Sphenodon*.

Lydekker, in part 2 of his *Catalogue of fossil reptiles in the British Museum* published in 1889, recognized the possibility of sub-dividing the genus *Ichthyosaurus* while Fraas (1891) figures many separate or displaced bones of the otic and occipital regions in a number of species. He gave good figures of the columella cranii, together with restorations.

The otic region was further discussed by Dollo (1907).

Merriam (1908) reviewed all the Triassic material of North America and compared it with European Triassic material, while Andrews (1910) and Sollas (1916) published detailed accounts of the cranial morphology of *Ophthalmosaurus* and *Ichthyosaurus* respectively.

Von Huene's (1916) account of *Mixosaurus* was followed in 1922 by his comprehensive work on Liassic ichthyosaurs, their classification and affinities with Triassic and post-Liassic species, and in 1949 he gave an account of the morphology of the posterior part of the skull of *Leptopterygius*.

Finally, Appleby (1956) described, inter alia, the otic and occipital regions of *Ophthalmosaurus*.

Recent work on ichthyosaur affinities is discussed in the penultimate section of this paper.

INTERPRETATION OF THE SKULL

The bones of the ichthyosaur skull are well known (Andrews, 1910; Sollas, 1916). *Ichthyosaurus* and *Ophthalmosaurus*, which have supplied the most information, represent the Jurassic latipinnate group, and *Leptopterygius* and

Stenopterygius, while contributing less information, represent the Jurassic longipinnate group. Further information has been drawn from unidentified Jurassic ichthyosaur material. The Triassic material is studied from the literature cited.

Occipital arch and foramen magnum

The occipital arch comprises the basioccipital, exoccipitals and supraoccipital and encloses the foramen magnum.

Basioccipital

The basioccipital carries the whole occipital condyle which is generally convex but which may be concave in some primitive forms e.g. *Cymbospondylus*. The position of the notochord is indicated by the presence of a small median pit. This is characteristic of all Jurassic ichthyosaurs. In Liassic forms the anterior end of the basioccipital fits closely into a corresponding hollow in the postero-dorsal side of the basisphenoid (L.C.M. 100'1949/146. See p. 370 for list of abbreviations of names of museums). There is no correspondence between these bones in *Ophthalmosaurus* (Fig. 1).

In Jurassic forms the exoccipital facets are clearly defined. Between them, in the floor of the foramen magnum and in the midline anterior to it, an area of finished bone descends to the anterior margin of the basioccipital (L.C.M. 100'1949/146). In Liassic forms finished areas on either side of the midline and anterior to the exoccipital facets are present and there is also a facet for the opisthotic on the anterolateral side of the basioccipital immediately below the exoccipital facet. This is difficult to discern in *Ophthalmosaurus* where it is less extensive ventrally.

Exoccipital

The exoccipital is slender in *Ichthyosaurus* (H.M.S.M. 84610, 84611) but stout in *Ophthalmosaurus* (L.C.M.100'1949/64; P.M. P.8). It is separated from the basioccipital by a pad of cartilage. In all Jurassic forms a foramen for the twelfth cranial nerve opens on the posterior surface and laterally the exoccipital takes part in the formation of the jugular, or vagus, foramen, through which emerge the tenth and eleventh cranial nerves and the jugular vein. There is also a small foramen for a branch of the eleventh or twelfth cranial nerve within the jugular foramen which passes anteriorly and medially into the endocranial cavity. In some Liassic ichthyosaurs a notch, possibly for a cranial nerve branch or a blood vessel, is present in the anterolateral margin of the bone (H.M.S.M. 84610, 84611).

Supraoccipital

The supraoccipital borders the foramen magnum dorsally and rests upon the exoccipitals. The ventral embayment is always fully ossified, and is shallow in most Liassic forms (H.M.S.M. 84621) giving a round foramen magnum, which is the primitive condition, but it is deep (advanced) in Ophthalmosauridae (L.C.M.100'1949/45) (Fig. 2).

Epiotic areas form the median dorsal bony wall of the vestibular part of the otic capsule. Median to these, two foramina penetrate the bone anteroposteriorly in all Jurassic genera. In Liassic forms a median foramen is present together with a variable number of blind foramina which are probably venous.

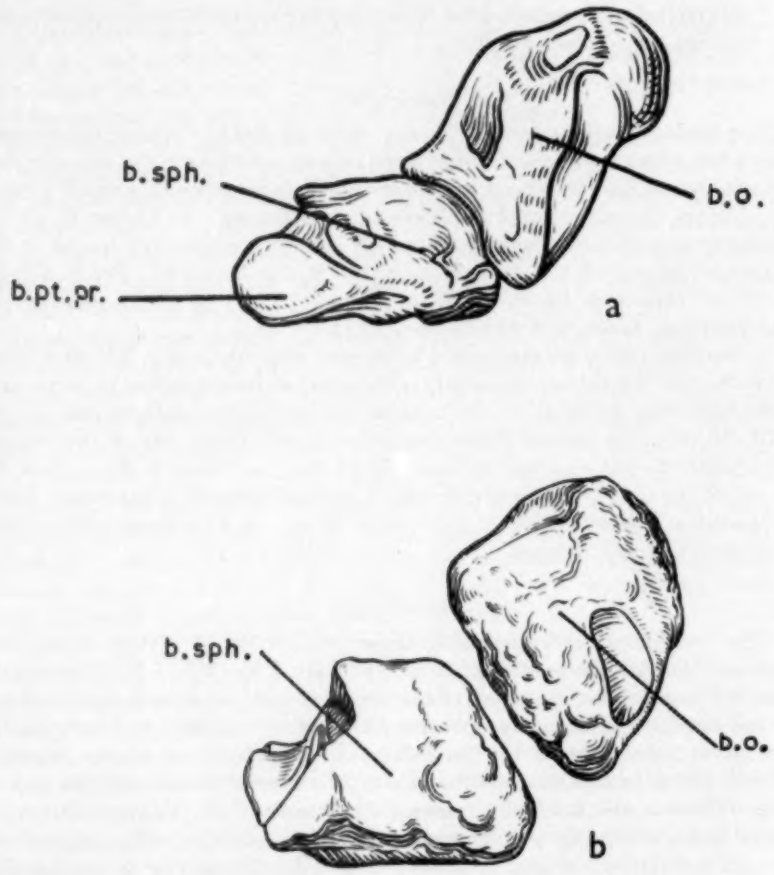


Fig. 1—Lateral views of the basioccipitals and basisphenoids of: (a) *Ichthyosaurus* (Leicester Museum 100'1949/146), (b) *Ophthalmosaurus* (Leicester Museum 100'1949/64). Brought to same length. (Key to lettering, p. 370.)

In all Jurassic forms ossification is rarely complete round the dorsal and lateral edges of the bone. The lateral extent of the cartilage was probably small, being limited by a post-temporal fenestra indicated by the area of finished bone on the whole dorsal surface of the opisthotic and the lateral surface of its dorsal process (L.C.M.100'1949/45).

Basisphenoid

The basisphenoid lies anterior to the basioccipital. In Jurassic forms the bone is wider than long with laterally directed basipterygoid processes and in *Mixosaurus* the bone is longer than wide with anterolaterally directed basipterygoid processes (Wiman, 1912).

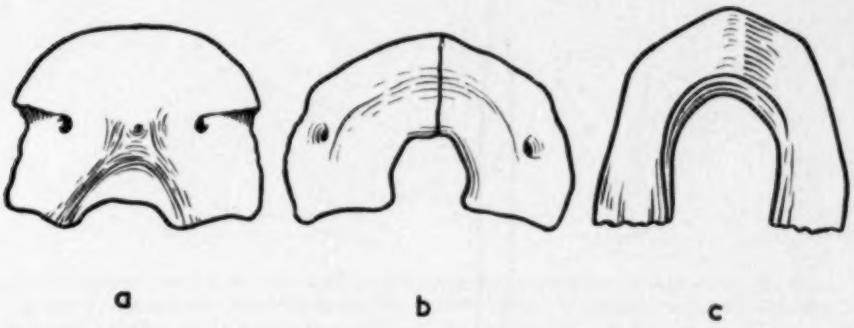


Fig. 2—Supraoccipitals of (a) *Ichthyosaurus* (H.M. Survey—No. 84621), (b) *Ophthalmosaurus* (Leicester Museum 100' 1949/45) and (c) *Baptanodon* (After Gilmore 1905). Brought to same width.

In all forms, particularly in *Ophthalmosaurus*, the bone is difficult to interpret, partly because of incomplete ossification, and also because only one foramen, for the carotids, is present. The carotid canal is a median structure and passes anterodorsally from an opening on the ventral side to an opening at the base of the large pituitary fossa. In most forms the ventral opening is single, but in one specimen of *Ichthypsaurus* s.l. sp. (L.C.M.100'1949/185) the opening is paired, and in other forms, two grooves on the ventral side converge on the single opening from the posterolateral corners of the bone (H.M.S.M. 84608). Where the opening is paired, the division is close to the ventral end of the foramen, and the piece of bone separating the openings may be parasphenoid. In L.C.M.100'1949/185 a trace of a suture is present posteriorly behind the paired openings. In *Mixosaurus* the proximal opening of the carotid foramen is more anteriorly placed than in the Jurassic genera.

In Jurassic ichthyosaurs roughened surfaces on either side of the midline on the anterior face of the basisphenoid, and more ventral than the pituitary fossa, are interpreted as junctions of anterior portions of trabecular cartilages. In *Mixosaurus*, although the roughened surfaces cannot be seen, a division on the ventral side between the proximal openings for the carotids and the anterior edge of the bone, suggest the presence of partly ossified trabecular cartilages.

In all Jurassic forms, two broad hollows, one on each side of the basisphenoid, converge anteriorly but remain lateral to the trabeculae. They pass posteriorly between the otic capsule and the quadrate wing of the pterygoid (i.e. cranioquadrate passage of Goodrich (1930)) then pass over the stapes. These are interpreted as the paths of the lateral head veins (Fig. 3). The posterior opening of the passage is large.

The absence of a groove or foramen for the sixth cranial nerve indicates that ossification on the dorsal side of the bone was very incomplete in all Jurassic genera.

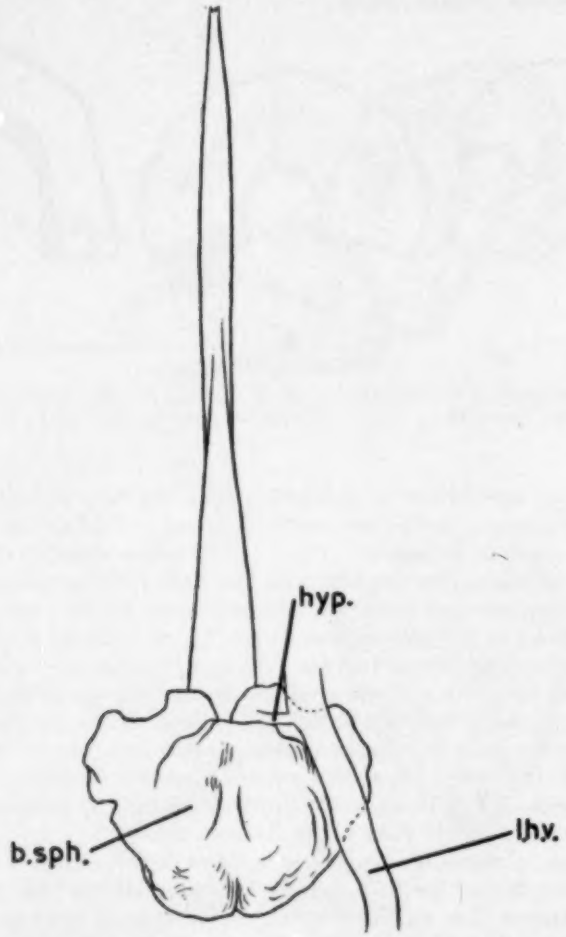


Fig. 3—Dorsal view of the basisphenoid of *Ophthalmosaurus* showing the course of the lateral head vein. × ½ approx. (Based upon Andrews, 1910.) (Key to lettering, p. 370.)

Two blunt processes on the anterodorsal edge of the basisphenoid on either side of the midline represent ossification in the bases of the pilae antotica and acrochordal cartilage.

In *Mixosaurus* a notch in the side of the bone may mark the path of the palatal branch of the seventh cranial nerve.

Palatoquadrate cartilage (Figs. 4 & 16)

The ossifications in the palatoquadrate cartilage are the epipterygoid (unknown in *Ophthalmosaurus*) and the quadrate. The position and shape of these two bones together with other information help to establish the extent of much of the palatoquadrate cartilage.

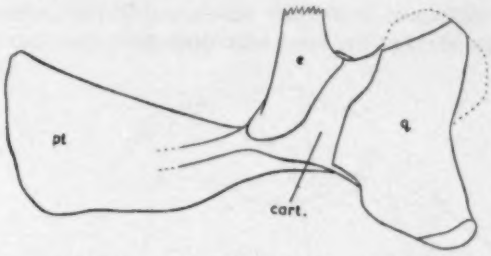


Fig. 4—Lateral view of the pterygoid, epipterygoid and quadrate in *Ichthyosaurus* (L. Lias), showing the relationships of elements and the inferred extent of the palatoquadrate cartilage. (Leicester 100'1949/111.) × ½ approx. (Key to lettering, p. 370.)

In *Ichthyosaurus* s.l. (L.C.M.100'1949/111) a sutural contact is present between the laterally placed epipterygoid and the more medially placed quadrate wing of the pterygoid, which is overlapped by a very small part of the foot of the epipterygoid (Fig. 4). The contact is at the level of, and in front of, the basipterygoid articulation. The epipterygoid is in a similar position in *I. intermedius* (B.M.N.H. R. 6697) and both differ from the position inferred by Sollas in *I. communis* (Sollas, 1916).

There are surfaces for the application of cartilage round the foot of the epipterygoid in all cases. The position of the quadrate is found by measurement in *Ichthyosaurus* s.l. (L.C.M.100'1949/111). It also has surfaces for the application of cartilage but there is little space between the two bones for the cartilage while in *I. intermedius* (B.M.N.H. R. 6697), which is smaller, there is virtually none.

In *Ichthyosaurus* s.l. (L.C.M.100'1949/111) a groove is present on the pterygoid, in which the quadrate rests posteriorly. It passes anteriorly and ventrally to the foot of the epipterygoid and fades out anterior to the basipterygoid articulation. This is interpreted as the ventrolateral limit of the palatoquadrate cartilage. In *I. intermedius* (B.M.N.H. R. 6697) the groove is small. Sollas' hollow on the pterygoid is also slightly anterior to the foot of the epipterygoid.

With the position of the epipterygoid known, it is possible to infer the position of the ganglion of the fifth cranial nerve, which is medial to it, the nerve roots passing out anteriorly and posteriorly. In *I. intermedius* (B.M.N.H. R. 6697) a notch at the foot of the epipterygoid may indicate the course of one of these branches.

Merriam's (1908) figures of *Cymbospondylus* suggest that the quadrate was sloping, as in primitive tetrapods, and was probably, therefore, in the primitive condition.

Otic capsule

Much cartilage was present between the opisthotic, prootic and epiotic region of the supraoccipital.

The positions of the bones are well known in *Ophthalmosaurus* (L.C.M. 100'1949/64) (Appleby, 1956), and are similar in *Ichthyosaurus intermedius* (B.M.N.H. R. 6697) and *I. communis* (S.M. J. 46924), and presumably in *Stenopterygius zetlandicus* (B.M.N.H. 32681 and 33157), where the opisthotic is similar in shape and has the same relationships to the basi- and exoccipitals.

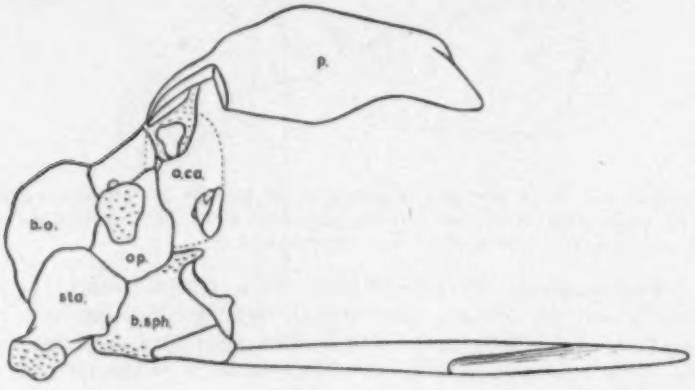


Fig. 5—Lateral view of the basicranial and otic regions of *Ophthalmosaurus* showing the approximate positions of the otic cartilages (Based upon Appleby, 1956). × ¼ approx. (Key to lettering, p. 370.)

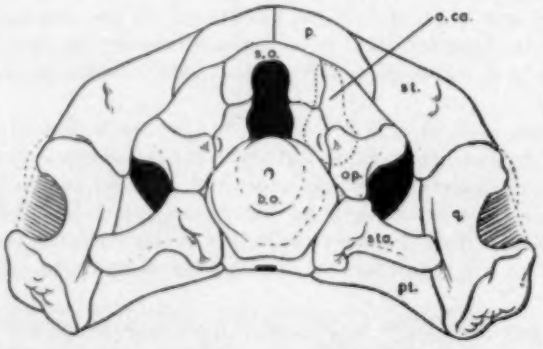


Fig. 6—Posterior view of the skull of *Ophthalmosaurus* showing the approximate position of the otic capsule (based upon Appleby, 1956). ×2/7 approx. (Key to lettering, p. 370.)

In *Ophthalmosaurus* the limits of the canalicular part of the otic capsule may be inferred by connecting the areas of bone which show cartilaginous roughening (Figs. 5, 6 & 7). The capsule probably bulged into the temporal muscle space and also into the endocranial cavity as shown by the ventromedial

course taken by the common canal and the direction of the impressions on the opisthotic. The internal auditory meatus is large in all forms and faces laterally. In *Ophthalmosaurus* (L.C.M.100'1949/64) a hollow on the anterior face of the prootic passes laterally from the endocranial cavity into the cranioquadrate passage. This is interpreted as the path of the seventh cranial nerve and indicates that the prootic ossification is entirely in capsular cartilage and not in prefacial commisure plus capsular cartilage. The absence of a foramen in the prootic in all forms supports this.

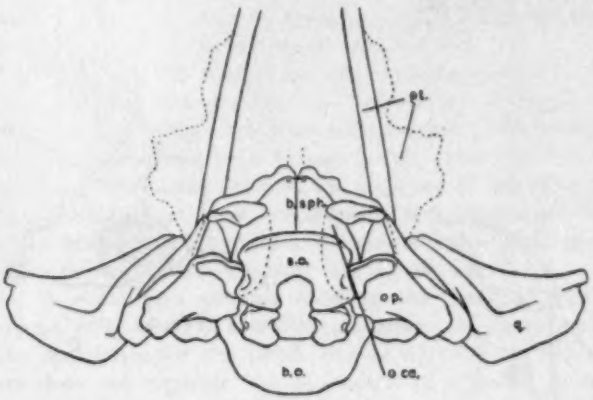


Fig. 7—Dorsal view of basicranial and otic region of *Ophthalmosaurus* showing the approximate positions of the otic cartilages (based upon Leicester Museum (100'1949/39, 100'1949/50, 100'1949/64, 100'1949/79). × 2/7 approx. (Key to lettering, p. 370.)

The canal system extends from just below the skull roof to just below the level of the braincase floor. In *Ophthalmosaurus* and *Ichthyosaurus*, and, presumably, in *Stenopterygius*, there is a cavity posterior and ventral to the canalicular part of the capsule, bounded by the pterygoid ventrally, finished areas of basicranium medially, the stapes posteriorly and posterolaterally and the lateral head vein anteriorly and anterolaterally. This is the saccular cavity. There is no bony partition between the saccular and canalicular cavities.

All Liassic opisthotics differ from the ophthalmosaurian opisthotic in that they have a posterior ventral process which carries part of the facet for the basioccipital posteriorly (Fig. 8). The loss of this process in *Ophthalmosaurus* is correlated with increase in the size of the head of the stapes (see p. 343).

In all ichthyosaurs the opisthotic bears a groove on the ventral surface. It passes from the endocranial cavity between the semi-circular canal system and the jugular foramen, runs posterolaterally beneath the opisthotic and reaches the cranioquadrate passage. The groove is closed ventrally by the head of the stapes which may show a corresponding groove. Because of these relationships it could not have transmitted the stapedial artery, the hymandibular branch of the seventh nerve or the lateral head vein. It is interpreted as marking the course of the ninth cranial nerve (Fig. 8, 1X).

The fenestra ovalis is difficult to identify because the capsule is poorly ossified and much modified but it seems certain that the ventral surface of the opisthotic posterior to the path of the ninth cranial nerve is not homologous with any part of it.

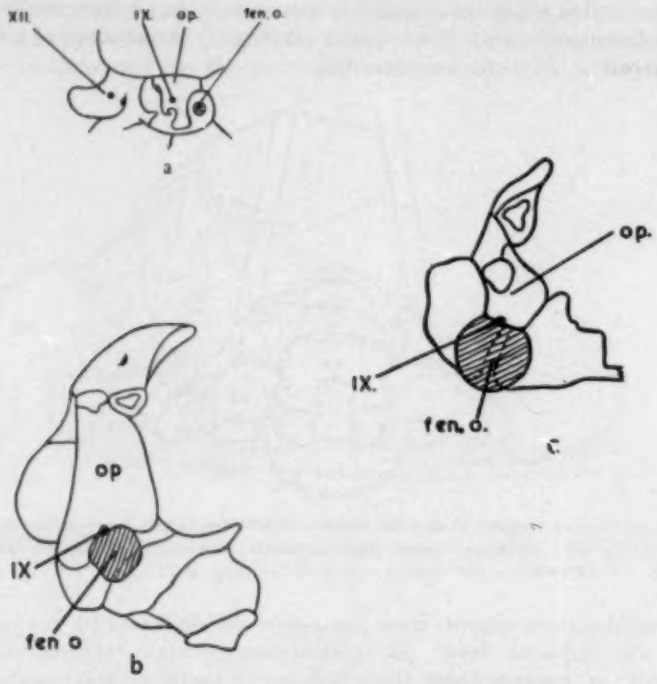


Fig. 8—Right lateral views of the otic regions of (a) *Chelone*, (b) *Ichthyosaurus* and (c) *Ophthalmosaurus*. Brought to approximately the same length. (Key to lettering, p. 370.)

In all Jurassic ichthyosaurs the paroccipital process of the opisthotic ascends dorsolaterally to meet the supratemporal. Apart from the contacts with the exoccipital, its dorsal edge is finished bone, denoting that the posttemporal fenestra was bounded ventrally by the whole length of the process and was probably relatively large. This gives the back of the skull of both lati- and longipinnate ichthyosaurs a very primitive aspect which is accentuated by the relatively depressed proportions.

Stapes

The stapes is known in the latipinnate ichthyosaurs *Mixosaurus* (von Huene, 1916), *Ichthyosaurus intermedius* (B.M.N.H. R. 6697), *I. communis* (S.M. J. 46924) and *Ophthalmosaurus* (Appleby, 1956), and among longipinnates it is known in *Cymbospondylus* (von Huene, 1916) and *Stenopterygius zetlandicus* (B.M.N.H. 32681 and 33157).

The stapes is large in *Ophthalmosaurus* (L.C.M.100'1949/64) where it is firmly held ventrally by the pterygoid, laterally by the quadrate and medially, via cartilage, by the basioccipital. No major blood vessels or nerves could pass beneath the stapes and there is no stapedial foramen. The head of the stapes is also relatively large in this genus and underlies the whole of the opisthotic (Fig. 8). The stapes is a simple necked rod-like structure with no processes.

The relationships of the bone are the same in *I. intermedius* and *I. communis* as in *Ophthalmosaurus*, except for a posterior ventral process of the opisthotic which descends slightly, posterior to the head of the stapes, which is smaller. The head of the stapes is even smaller in *Mixosaurus*.

In longipinnate forms the head of the stapes increases between *Cymbospondylus*, from the Middle Triassic, and the Liassic *Stenopterygius zetlandicus*. In general, Lower Liassic stapes heads are intermediate in size between Triassic ones and Upper Jurassic or Upper Liassic forms. (See Fig. 9.)

The increase of the stapes head at the expense of the posterior ventral process of the opisthotic, in both longipinnate and latipinnate ichthyosaurs suggests that, in primitive ichthyosaurs, the stapes had a relatively small head and the opisthotic had a relatively large ventral process.

A groove is sometimes present crossing the head of the stapes in *Ophthalmosaurus*, *Ichthyosaurus* and *Stenopterygius*. It corresponds with the groove in the opisthotic for the ninth cranial nerve (Otic capsule, p. 341), and therefore does not separate the equivalent of a dorsal process from a footplate.

In *Ophthalmosaurus*, another groove passes anterodorsally and laterally across the posterior side of the head of the stapes. There is no stapedial foramen and nothing could have passed beneath the stapes. Therefore the groove is interpreted as the path of the stapedial artery.

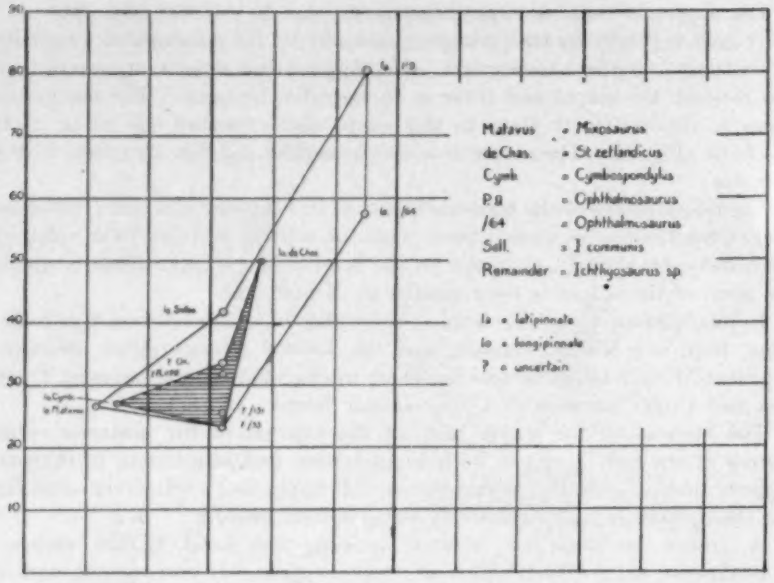
All Jurassic ichthyosaurs have a roughening, median to the middle of the stapes on the posterior side, suggesting the origin of a hyoid ligament connecting with the ventrolaterally placed hyoid elements. There is no otic notch in ichthyosaurs.

Presphenoid region

The chondrocranium anterior to the basisphenoid is completely unossified in ichthyosaurs.

In *Ichthyosaurus communis* (O.U.M. J. 2239/p) the left and right sides of the skull roof meet in a single contact, whereas in *Ophthalmosaurus* (P.M. P.8) the two sides meet and turn down in the midline (Fig. 10).

In all forms, hollows pass from the region of the pineal opening to a point immediately posterior to the external nasal opening, on either side of the midline. The lateral limit of the hollows is a ridge passing from the anterior end of the supratemporal fossa to the converging sides of the skull on the anterodorsal margin of the orbit. This ridge has different compositions in *I. communis* and *Ophthalmosaurus*. In the latter the prefrontal extends on the ventral side of the skull roof as far as the supratemporal fenestra, while in *I. communis* it finishes a little way anterior to the opening (Fig. 11).



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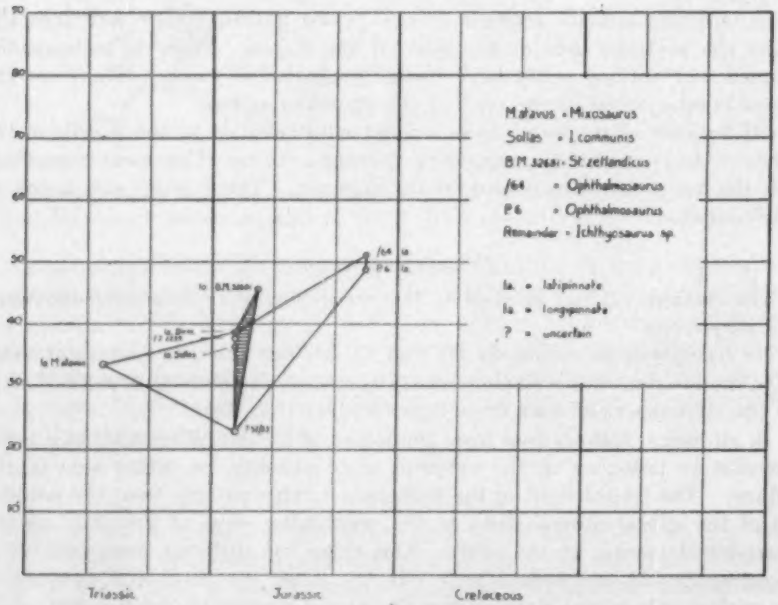

b

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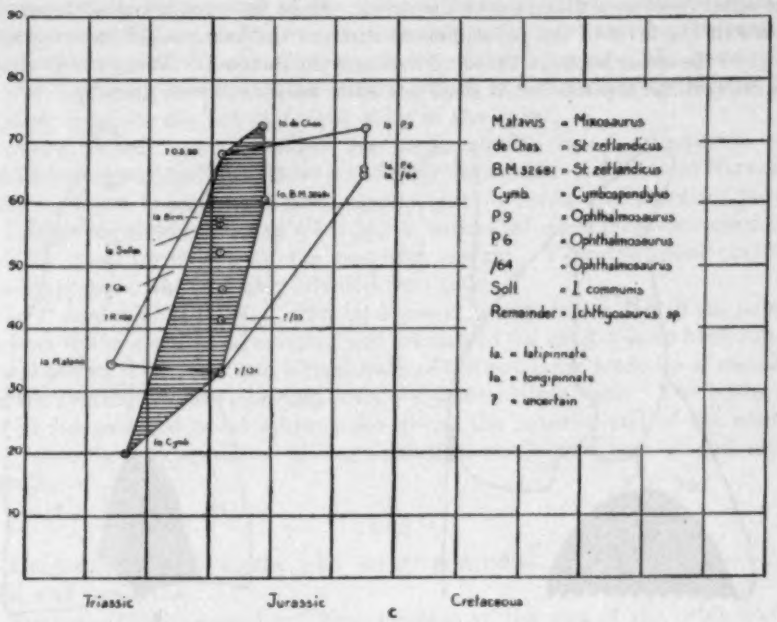

Fig. 9—Changes of size of the head of the stapes expressed as (a) percentage of the width of the basioccipital, (b) percentage of the width of the basisphenoid at the basipterygoid articulation, and (c) percentage of the length of the stapes.

Lateral to these ridges in all forms two shelves of bone made up of pre- and postfrontal form the dorsal borders of the orbit. An uncrushed specimen of *Ichthyosaurus* sp. (L.C.M.100'1949/164) indicates that the sclerotic rings underlay these shelves and a specimen in the Manchester Museum, which shows much of the sclerotic rings, suggests that the eye was disc shaped and only bulged in slightly, leaving the brain but little constricted at this point.

The palate of Jurassic ichthyosaurs was strongly arched (*Ichthyosaurus* sp., L.C.M.100'1949/112); *Ophthalmosaurus* sp., L.C.M.100'1949/64). The universal presence of a parasphenoid rostrum in ichthyosaurs suggests that a median septum was present. Even if the septum was low, the arching of the palate would bring its dorsal edge, and hence the ventral side of the brain, level with

Fig. 10—Sections through the skull roof of (a) *Ichthyosaurus* and (b) *Ophthalmosaurus*. (Brought to approximately the same width.)

the anteroposterior diameter of the oribt. Since the eyes are closest together at about the level of the parasphenoid rostrum the brain could have occupied most of the space between the skull roof and the rostrum. There was probably an interorbital septum but it need not have been extensive dorsally.

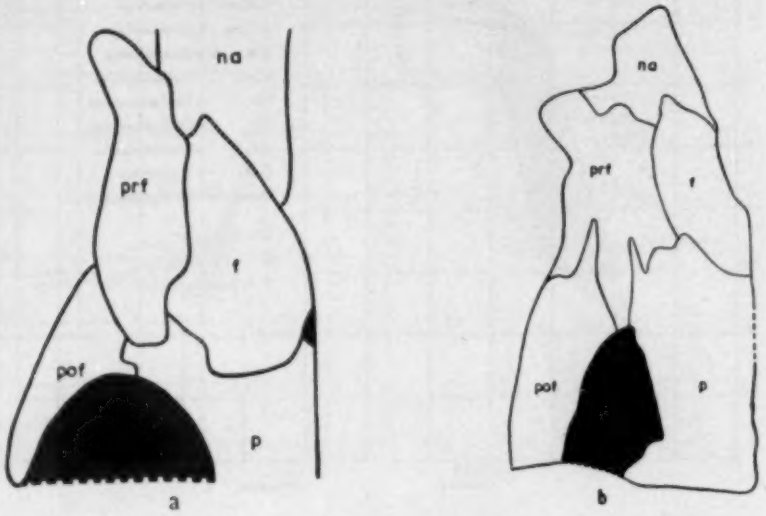

Fig. 11—The roof of the skull in the fronto-temporal region in ichthyosaurs, in ventral view, showing the limits of the prefrontal. (a) *Ichthyosaurus* (after Sollas, 1916), and (b) *Ophthalmosaurus* (after Peterborough, P. 8).

Nasal capsule

The form of the nasal capsule may be partially inferred from the shapes of adjacent dermal bones.

In *Ichthyosaurus communis* (O.U.M. J. 2239/p) (Fig. 12) and *Ophthalmosaurus* (P.M. P. 8) the hollows on the underside of the skull roof terminate

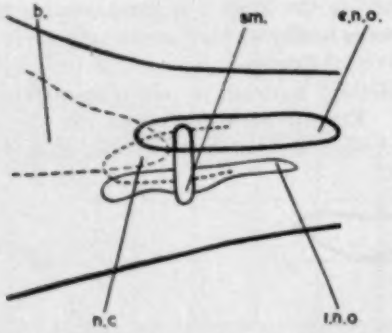

Fig. 12—The nasal region of *Ichthyosaurus communis* with dorsal and lateral elements removed and with part of the anterior end of the endocranial cavity restored. (After Oxford University Museum J.2239/p.)

dorsal to the posterior end of the internal nasal opening. Ventromedially, corresponding hollows on the vomers fade anteriorly approximately below this point. These two sets of hollows mark the anterior limits of the brain.

The ascending lamellae of the vomers, which lie apart on either side of the midline, separate the left and right sides of the brain.

Sollas (1916) has identified the septomaxilla in *I. communis*. In *Ophthalmosaurus* (Andrews, 1910) a ventrally directed projection from the nasal tends to divide the external nasal opening into anterior and posterior parts. In *Ichthyosaurus crassimanus* (M.U.M.) a horizontal shelf projects anteriorly into the nasal opening from the posterior margin. Thus the nasal opening in ichthyosaurs was probably divided into two.

In *I. communis* (O.U.M. J. 2239/p) a groove on the dorsal part of the palate between the internal nasal opening and the side of the skull, passes back into a lateral recess. The floor and lateral walls of the recess are made up of maxilla, and the prefrontal and lachrymal enter the posterolateral wall. The posterior end of the external nasal opening lies above the anterior end of the recess. The recess is interpreted as having contained the lateral part of the nasal capsule.

Orbit

The bones surrounding the orbit are the prefrontal, postfrontal, postorbital, jugal and lachrymal.

Merriam (1908) pointed out that increase in the size of the orbit was a general ichthyosaur feature and to establish this, proportionate change was studied in three ichthyosaur lines of descent (*Mixosaurus atavus* to *M. cornalianus*; *Ichthyosaurus* to *Ophthalmosaurus* and *Cymbospondylus* to *Leptopterygius*). (Phylogeny according to von Huene, 1922 and 1955).

Because the relative length of the snout varies with size in a given species of ichthyosaur, the reference length in terms of which other dimensions could be expressed was taken as the total postnasal length of the skull. The percentages were plotted against a stratigraphical time scale (Fig. 13).

The anteroposterior diameter of the orbit was measured and plotted and was found to increase in all three lines (Fig. 13a, A and 13b, A). By measurement of the cheek region posterior to the orbit (Fig. 13a, C and 13b, C) and the region between the orbit and the external nasal opening (Fig. 13a, B and 13b, B) it was shown that expansion of the orbit was at the expense of the cheek region. As this went on the bones of the cheek region became narrower while the bones above and below the orbit became elongated. The external exposure of the postfrontal is larger than that of the prefrontal in the later members of each of the three lines of descent and the jugal also becomes a very elongate bone.

Primitively ichthyosaurs would have small anteriorly placed orbits with wide cheeks and would have pre- and postfrontals and jugals of more conventional size.

In *Ophthalmosaurus* the orbit is at a maximum leaving little bone in the side of the skull in the orbital region. Since the snout is also extremely elongate the enlarged orbit leads to mechanical weakness in the skull. This may be

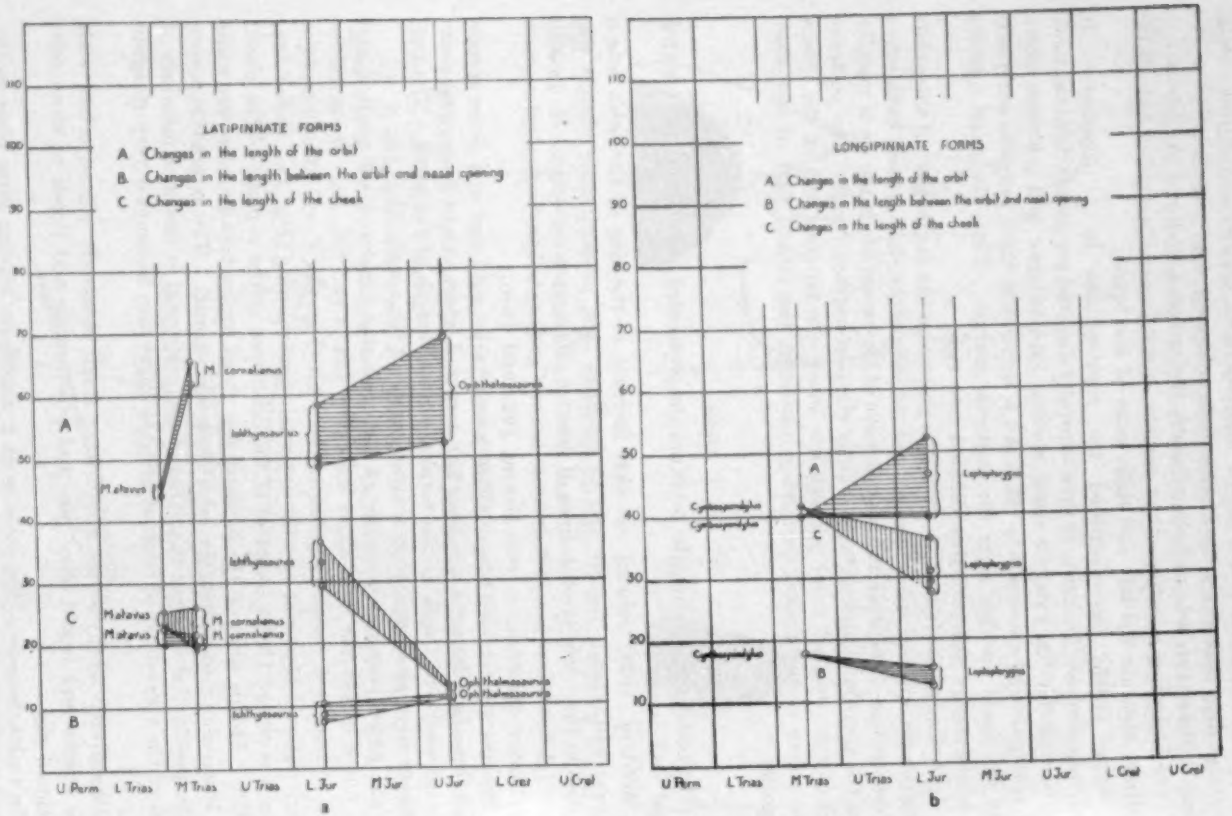

Fig. 13—Graphs illustrating the changes in length of the orbit in ichthyosaurs.

compensated for by a number of anteroposteriorly directed features, such as the large posterior extent of the prefrontal on the ventral side of the skull roof and the downturning of the bones of the skull roof as they meet in the midline (p. 343). The ascending lamellae of the vomers in this line of descent

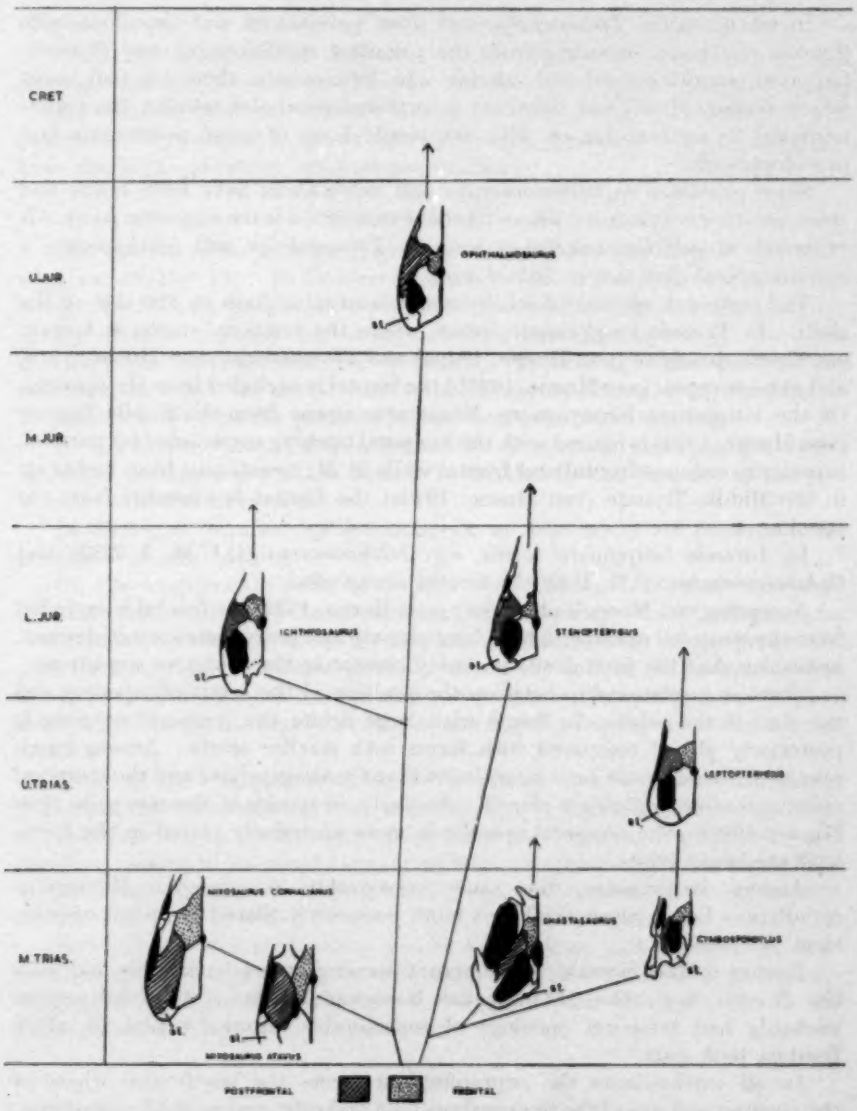

Fig. 14—The temporal region in ichthyosaurs.

(p. 347) may also be a strengthening device. These features would be absent or poorly developed in primitive ichthyosaurs. The strongly interdigitating sutures of the dermal roofing bones of *Ophthalmosaurus*, *Ichthyosaurus*, etc. are also probably a partial compensation for mechanical weakness.

Temporal region (Fig. 14)

In ichthyosaurs, *Triassochelys* and most pareiasaurs and procolophonids there is one bone remaining from the primitive reptiliomorph row of intertemporal, supratemporal and tabular. In *Nannopareia* there are two bones which Watson (1957) has identified as supratemporal and tabular, the supratemporal being homologous with the single bone of most pareiasaurs and procolophonids.

Since primitive captorhinomorphs and pelycosaurs have both bones and since, in other cotylosaurs, where one bone remains, it is the supratemporal, it is proposed to call the remaining bone in *Triassochelys* and ichthyosaurs a supratemporal (but see p. 362 *et seq.*).

The temporal opening of ichthyosaurs is situated high on the side of the skull. In Triassic longipinnate forms, where the temporal region is known, e.g. *Cymbospondylus* (von Huene, 1949a) and *Shastasaurus* (von Huene, 1916) and *Stenopterygius* (von Huene, 1949b) the frontal is excluded from the opening. Of the latipinnate ichthyosaurs, *Mixosaurus atavus* from the Middle Triassic (von Huene, 1935) is figured with the temporal opening surrounded by parietal, supratemporal, postfrontal and frontal while in *M. cornalianus* from higher up in the Middle Triassic (von Huene, 1949a) the frontal is excluded from the opening.

In Jurassic latipinnate forms, e.g. *Ichthyosaurus* (O.U.M. J. 2293) and *Ophthalmosaurus* (P.M. P.8), the frontal is excluded.

Accepting von Huene's phylogeny (von Huene, 1922) the frontal is excluded from the temporal opening in both longipinnate and latipinnate lines of descent, indicating that the frontal was probably present in the primitive condition.

There is a relationship between the position of the temporal opening and the size of the orbit. In forms with large orbits the temporal opening is posteriorly placed compared with forms with smaller orbits. Among longipinnates, *Shastasaurus* has a larger orbit than *Cymbospondylus* and the temporal opening is more posteriorly placed. Similarly, in species of *Stenopterygius* (von Huene, 1949b), the temporal opening is more posteriorly placed in the forms with the larger orbits.

Among latipinnates, the same relationship is present. *Mixosaurus cornalianus* has a larger orbit and more posteriorly placed temporal opening than *M. atavus*.

During orbital increase the temporal opening moves backwards and loses the frontal from the anteromedial boundary. Primitively, ichthyosaurs probably had temporal openings of considerable anterior extent in which frontals took part.

In all ichthyosaurs the supratemporal meets the postfrontal whatever the position and size of the temporal opening and orbit and is, in all probability, a primitive ichthyosaur character.

In Jurassic forms the prefrontal is excluded from contact with the frontal by the meeting of the nasal with the postfrontal. In Triassic forms the frontal, prefrontal and postfrontal meet at a point, which is the more primitive condition.

The interparietal is universally absent in ichthyosaurs.

Pineal foramen

With the exception of *Grippia* (Wiman, 1928), the pineal foramen of ichthyosaurs lies between the frontals and the parietals in the midline. In *Grippia* the foramen lies entirely in the parietals, as in most amphibia and reptiles. The presence of the primitive tetrapod condition in *Grippia* suggests that this is the primitive ichthyosaur condition.

Nasal opening (Fig. 15)

The pattern of the bones surrounding the nasal opening in ichthyosaurs is very varied (Fig. 15). In Triassic latipinnates, i.e. *Mixosaurus*, there are two conditions. In the earlier *M. atavus* (von Huene, 1935) an ascending process of the maxilla excludes the lachrymal from the nasal opening. The premaxilla is just excluded from the opening by the meeting of the nasal and the maxilla anteriorly. In the later *M. cornalianus* (von Huene, 1956) the position is similar except that the premaxilla just enters the nasal opening.

In the Lower Jurassic *Ichthyosaurus* the premaxilla, nasal and lachrymal surround the opening and the maxilla is exluded by the junction of the premaxilla with the lachrymal.

In the Upper Jurassic *Ophthalmosaurus*, all four bones are present in the boundary of the opening.

On the longipinnate side, in *Grippia* (von Huene, 1956) the lachrymal, maxilla, premaxilla and nasal surround the opening. In *Cymbospondylus* (von Huene, 1949a) the nasal is excluded by the junction of the premaxilla with the lachrymal. *Leptopterygius* (Piveteau, 1955), from the Lower Jurassic, is identical with *Ichthyosaurus* in this region but in *Stenopterygius* (Piveteau, 1955) a fifth bone, the jugal, is present, meeting the premaxilla to exclude the maxilla.

Thus, on the longipinnate side, the lachrymal and premaxilla are always present in the opening, and, with the exception of *Cymbospondylus*, the nasal is always present. On the latipinnate side, the lachrymal, premaxilla and nasal are present in the nasal opening with the exception of *Mixosaurus*, where the lachrymal is excluded in both species and the premaxilla just excluded in *M. atavus*.

Although no firm conclusions can be drawn from this distribution, it is probable that the lachrymal, maxilla, premaxilla and nasal were primitively present, as in *Grippia* and primitive tetrapods while the exclusion of the lachrymal is a *Mixosaurus* specialisation.

Palate

The dermal bones of the palate are the premaxilla, maxilla, vomer, palatine, pterygoid and parasphenoid. Merriam (1908) suggested that the ectopterygoid

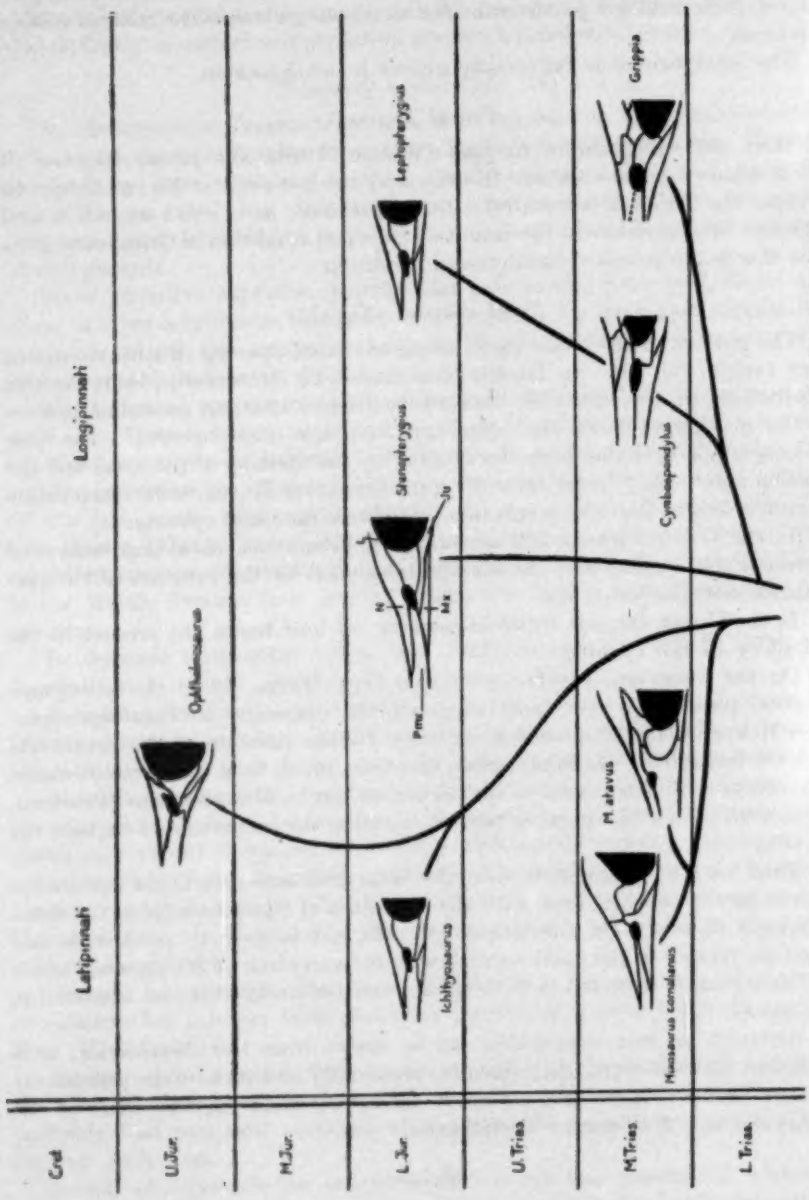

Fig. 15—The external nasal opening in ichthyosaurs (Diagrams after Piveteau, 1958).

may have been present in *Cymbospondylus* where it took part in the boundary of the subtemporal fenestra.

The interpterygoid vacuities vary considerably between Triassic and Jurassic forms, and where possible, the maximum width of the vacuities has been measured in terms of the total width of the palate at the same point. On the latipinnate side, the vacuities of *M. cornalianus* (von Huene, 1916) are a fourteenth of the total width, and in *Ichthyosaurus* (Sollas, 1916) and *Baptanodon* (Gilmore, 1905) they are a third. On the longipinnate side, the vacuities of *Cymbospondylus* (Merriam, 1908) are a sixth of the total width, while in *Leptopterygius* (von Huene, 1956) and *Stenopterygius* (von Huene, 1922) they are a third. The vacuities of *Shastasaurus* (Merriam, 1902b) and *Omphalosaurus* (Merriam, 1906) from the Middle Triassic are respectively a quarter and a fifth.

These proportions show that there is a general increase in width from the Triassic to the Jurassic on both latipinnate and longipinnate sides. Primitively ichthyosaurs probably had narrow interpterygoid vacuities.

The proximal end of the parasphenoid rostrum occupies more or less of the interval between the basipterygoid processes. Among latipinnates the parasphenoid rostrum in *M. atavus* (von Huene, 1916) occupies the whole space between the basipterygoid processes while in *Ichthyosaurus* (Sollas, 1916) and *Ophthalmosaurus* (Andrews, 1910) the rostrum is narrow and occupies only a small part of the interval between the processes. On the longipinnate side the condition is uncertain but in *Shastasaurus* (Merriam, 1902b) the base of the rostrum is wide and tapers rapidly to a point. In *Leptopterygius* (von Huene, 1922) and *Stenopterygius* (von Huene, 1922) the condition is similar to *Ichthyosaurus* (Sollas, 1916).

Primitively the parasphenoid rostrum was probably broad based filling much of the space between the basipterigoid processes.

The suture between the anterior ends of the pterygoids is longer in *Cymbospondylus* (Merriam, 1908), *Shastasaurus* (Merriam, 1902b) and *Omphalosaurus* (Merriam, 1906) than in *Mixosaurus* or any of the Jurassic ichthyosaurs, i.e. the interpterygoid vacuities did not extend so far forwards in primitive ichthyosaurs.

There is no descending flange of the pterygoid in ichthyosaurs.

The position of the quadrate ramus of the pterygoid relative to the basipterygoid process is unknown in Triassic forms but, in the latipinnate *Ichthyosaurus* (B.M.N.H. R. 6697) and the longipinnate *Leptopterygius* (L.C.M.100'1949/111) it arises anterior to the process, which, if it is a general ichthyosaur feature, is a striking similarity to millerosaurs.

Dentition

With the possible exception of *Omphalosaurus* from the Middle Triassic (Merriam, 1906), the lateral teeth of ichthyosaurs are plicated and labyrinthodont. Domed palatal teeth occur only among Triassic longipinnates, e.g. *Pessopteryx* and *Grippia* (Wiman, 1910, 1928 and 1933). *Omphalosaurus* has domed teeth on the broad flat dorsal surface of the dentary which would be opposed, in life, to the palatal surface which presumably also carried teeth.

Conical thecodont marginal teeth occur in both lati- and longipinnate sides in *Mixosaurus* and *Cymbospondylus* and probably also in *Grippia*. In *Merriamia* (Merriam, 1903) and *Shastasaurus* (Merriam, 1908) the teeth were lodged in grooves, not sockets. Tooth differentiation also occurs on both sides in *Mixosaurus*, where it is extreme, in *Merriamia*, and, to a lesser extent, in *Cymbospondylus*.

A variety of tooth structures occurs in Jurassic and Cretaceous forms including carinate teeth (*Macropterygius*), curved teeth (*Stenopterygius*), smooth crowned teeth (*Leptopterygius*) and teeth with cement covering the roots (*Leptopterygius*).

The primitive ichthyosaurs probably had thecodont, conical and strongly fluted labyrinthodont, marginal teeth with little cement covering the roots and there *may* have been palatal dentition.

SOME RECENT THEORIES ON ORIGINS OF ICHTHYOSAURS

Several theories on the origins of ichthyosaurs have been advanced in recent years by Broom, Williston, Olson, von Huene, Nielsen, Romer and Watson. The two main streams of recent thought are contributed by von Huene and Nielsen on the one hand, and by Romer and Watson on the other. Broom (1924) and Williston (1925) classified ichthyosaurs with plesiosaurs and protorosaurs on their common possession of an upper temporal opening. This theory may be rejected on the grounds that the bones surrounding the opening in ichthyosaurs (p. 350) differ from those in plesiosaurs and protorosaurs. The fact that two groups have temporal openings in similar positions is insufficient evidence in itself because openings in identical positions are found in unrelated groups, e.g. *Bolosaurus* and pelycosaurs (Watson, 1954).

Olson (1936) in discussing the affinities of *Youngina* implied that ichthyosaurs were close to both that genus and to the Squamata and Rhynchocephalia. He homologized the bone between the basicranium and the palate at the basipterygoid articulation in *Youngina* with the meniscus pterygoideus of lizards and, in a footnote, suggested that the anterior columella cranii of ichthyosaurs was also homologous. Since the anterior columella cranii of ichthyosaurs (i.e. *I. communis* (Sollas, 1916)) is the prootic, this possible homology is unfounded.

Von Huene (1922) placed ichthyosaurs with *Mesosaurus* but rejected this classification in 1937 when he derived them from embolomerous amphibia. Similarities to *Mesosaurus* were shown to be due to aquatic adaptation. *Mesosaurus* was placed with pelycosaurs.

The derivation of ichthyosaurs from embolomerous amphibia was based largely on the similarities of features which are primitive but which are also often seen in various reptile groups. These primitive features include the shape of the basicranium in ventral view, the long parasphenoid rostrum dividing the interpterygoid space, the concave occipital condyle and possibly the labyrinthodont teeth.

Von Huene lists as ichthyosaurian advances over the primitive embolomerous condition (1) the presence of the temporal opening, (2) the anterior

position of the pineal foramen between the frontals and the parietals, and (3) the possession of an anterior columella cranii.

Von Huene was in error concerning the pineal opening, which is, in primitive ichthyosaurs, of primitive tetrapod type (p. 351). The so-called "anterior columella" is the prootic and its presence therefore cannot be looked upon as an advance. The unique temporal opening is, indeed, an advance but it is one which has no parallel in any other group and is thus evidence of the independent origin of ichthyosaurs in anapsids.

In 1944 von Huene derived ichthyosaurs from loxommids giving few reasons. The peculiar temporal opening was used to derive ichthyosaurs from anapsids.

Von Huene (1946) again derived ichthyosaurs from Loxembolomeri listing as similarities many of the features used in von Huene (1937). New evidence given was the morphology of the basioccipital and basisphenoid and their strong separation. This separation is less marked in earlier ichthyosaurs than in later ones (p. 335) and is also present to a greater or lesser extent in *Ophiacodon*, *Diadectes* and other primitive reptiles.

In 1952a von Huene proposed a derivation of ichthyosaurs from the reptiliomorph ancestor of Diadectomorpha, Chelonia and Microsauria based upon postcranial features (gastrocentral vertebrae with haemal arches in the tail) and the unique temporal opening, which can only be derived from an anapsid condition. The large number of primitive characters was used to show that ichthyosaurs probably had their origin in Carboniferous times. Advances made by ichthyosaurs were listed including the heavy stapes meeting the quadrate which was without an otic notch.

Nielsen (1954) related ichthyosaurs to forerunners of trematosaurs through the newly described genus *Tupilakosaurus*. The relationship was based on the supposed possession by ichthyosaurs of a batrachomorph skull table, supported by the elongate compressed skull, labyrinthodont teeth, grooves suggesting a lateral line system, and their probable anapsid ancestry.

The supposed batrachomorph nature of the ichthyosaurian skull is based upon one specimen of *Leptopterygius* (von Huene, 1949b) in which the occipital flange of the parietal is shown with a suture separating it from the parietal for a short distance on either side of the midline before fading out. The position of the "suture" coincides with the irregular ridge, which is usually roughened, separating the occipital flange of the parietal from the body of the bone. This region has been examined in many Liassic and Oxford Clay specimens, including well preserved juvenile specimens extracted from limestone by acetic acid techniques but no suture has been observed. Since cracks occur in this region, the "suture" shown by von Huene (1949b) may also be cracks. If so, there is no evidence for the batrachomorph skull table in ichthyosaurs.

The grooves on the snout which Nielsen equates with lateral line grooves are very variable and ramify in a way reminiscent of blood vessels and do not possess the clear-cut channels of typical amphibian lateral line canals.

With the removal of the two main pieces of evidence, the remaining characters indicate primitiveness, reptiliomorph affinities and aquatic adaptation.

Von Huene (1956) followed Nielsen in relating ichthyosaurs to Batrachomorpha through *Tupilakosaurus*. The principal piece of evidence given was

the character of the posterior skull table, dealt with above (p. 355). Long interpterygoid vacuities were mentioned but it is shown above that these are a secondary development within the ichthyosaurs (p. 353). The suture between the supratemporal and squamosal was also claimed to be batrachomorph but this feature is probably due to the acquisition of strong interdigitating sutures by the outer dermal bones of the skull, which is correlated with orbital enlargement and increase in the length of the snout (p. 350).

The remainder of the characters is not relevant, being primitive or aquatic, e.g. labyrinthodont teeth, presence of a septomaxilla, presence of a hollow occipital condyle, elongation of the snout and posterior position of the nares.

The work of Nielsen and von Huene only shows that ichthyosaurs are descendents of primitive reptiliomorphs or reptiles and not of batrachomorphs.

Romer (1948) discussed and rejected earlier theories of ichthyosaur origins. He compared his own restoration of *Ophthalmosaurus* with *Ophiacodon* and concluded that though there were many similarities between the two animals, the differing structures of the temporal regions excluded *Ophiacodon* from direct ancestry of ichthyosaurs. He suggested an anapsid "pre-ophiacodont" as an ideal ichthyosaur ancestor.

Romer found that most of the similarities between the two genera were either primitive or aquatic but a small number were not.

Primitive characters shared included the bracing by the opisthotic of the bone which, in *Ophthalmosaurus*, he suggested might be the tabular, the thecodont labyrinthodont teeth and the small size and lateral position of the posttemporal fenestra. Romer was mistaken concerning the latter point. In ichthyosaurs the fenestra is larger and extends more medially (p. 342).

Similar aquatic features included the reduced ossification, elongation of the skull, the long tooth row and the small prootics.

The remaining similarities included the supposed presence of an unossified area of the supraoccipital bordering the foramen magnum. This feature is not present. In no case is a surface for the application of cartilage present and the dorsoventrally elongate foramen magnum is a specialisation which appeared within ichthyosaurs, e.g. *Ophthalmosaurus*, Liassic species having almost round foramina (p. 335). The large size of the stapes was also given as a similarity but this is only true of later, more advanced, ichthyosaurs. Primitive and early ichthyosaurs have relatively smaller stapes (p. 343). Another character of the stapes given by Romer was the inferred presence of a stout dorsal process separated from the head of the stapes by a groove. This groove is for the ninth cranial nerve (p. 343) and cannot therefore separate a dorsal process from a head of the stapes. The only real similarity to ophiacodonts is the slight lateral extension of the supraoccipital cartilage, a feature also seen in millerosaurs, *Sphenodon* and *Limnoscelis*.

Thus, there is no important reason for placing ichthyosaurs with primitive or pre-ophiacodonts and a number of real differences between the two groups supports this, e.g. the absence of a tabular, the meeting of the supratemporal with the postfrontal and the presence of an upper temporal opening.

Watson (1954) stated that ichthyosaurs are theropsid (based on the ear), a view he still held in 1957. Since, in ichthyosaurs, there is no dorsal process,

stapedial foramen or interval for cartilage between the distal end of the stapes and the quadrate (p. 343), ichthyosaur and "theropsid" middle ears are fundamentally different (see p. 359). Primitive ichthyosaur stapes are also smaller than those of later forms, (p. 343). "Theropsid" characters are also seen in *Nyctiphruretus*, where a simple rod-like stapes meets the quadrate, and in *Bolosaurus*, which lacks an otic notch.

The above theories of ichthyosaur origins are thus seen to be insufficiently supported and a new attempt to trace their origins is made below.

ICHTHYOSAUR ORIGINS

Ichthyosaurs are clearly derived from primitive reptiles or amphibia. This is shown by the primitive characters which are either present in the group or deduced to be present in the ancestral ichthyosaurs. Such characters include the relationship between the frontals, prefrontals and postfrontals (p. 351), the pattern of the bones surrounding the nasal opening (p. 351), the possible presence of palatal teeth (p. 354), the small to medium sized interpterygoid vacuities (p. 353), the proportions of the back of the skull (p. 342), the dorsolaterally ascending paroccipital processes (p. 342), the presence of cartilage in the braincase region (p. 337), the lack of a strong bony division between the semicircular canal system and the saccular region (p. 341) and the sloping quadrate (p. 339).

Ichthyosaurs were already advanced in a number of ways by Lower Triassic times. Chief among these advances were the presence of an upper temporal opening of unique construction (p. 350), the meeting of the supratemporal with the postfrontal (p. 350), the loss of the interparietal (p. 351) and tabular (p. 350), the presence in the occipital condyle of only one bone, the basioccipital (p. 335), the enlargement of the cranioquadrate passage (p. 337), the slight constriction of the endocranial cavity by the otic capsules (pp. 340 & 341), the ventral position of the saccule with respect to the floor of the endocranial cavity (p. 341), the posteroventral position of the saccule with respect to the semi-circular canal system (p. 341), the separate exit of the ninth cranial nerve from the endocranial cavity (p. 341), the path of the ninth cranial nerve across the capsule (p. 341), the loss of processes and stapedial foramen from the stapes (p. 343) and the loss of the otic notch (p. 343).

The presence of a unique upper temporal opening in ichthyosaurs suggests that the condition was derived from an anapsid condition and this at once eliminates a large number of reptile groups from ichthyosaurian ancestry. The large number of primitive tetrapod characters supports this.

The advanced characters were searched for among anapsid reptiles and amphibia. An almost complete correspondence was found with the Chelonia as represented by *Triassochelys* (Jaekel, 1918) and, where information in that was lacking, with later chelonians (*Chelone*). *Triassochelys* is anapsid but has a similar temporal region, in that the supratemporal meets the postfrontal, and the interparietal and tabular are absent. The occipital condyle is entirely basioccipital, a large cranioquadrate passage is present, the quadrate is exposed posteriorly and a separate opening in front of the jugular foramen may be interpreted as an opening for the ninth cranial nerve. Features which could

not be checked in *Triassochelys* but which are present as general modern chelonian features (de Beer, 1937) include the constriction of the braincase by the otic capsules, the ventral position of the saccule with respect to the floor of the endocranial cavity and its posteroventral position with respect to the semicircular canal system, the course of the ninth cranial nerve along a path separate from that of the tenth and crossing the capsule, and the loss of processes and the stapedial foramen from the stapes.

The most primitive known chelonians are already too specialized to be ancestors of ichthyosaurs. The shortening of the snout, loss of the pineal opening, raising of the skull roof on a parietal crest and the almost horizontal position of the paroccipital processes show this.

The presence of such a large assemblage of advances in the two groups of such different adaptations implies a common genetical origin rather than convergence due to similar modes of life.

The common ancestry of ichthyosaurs and chelonians must therefore antedate the first recorded appearances of the two groups. Ichthyosaurs are first recorded from the Lower-Middle Triassic boundary while undoubted chelonians make their appearance in the Upper Triassic. Their common ancestry would thus be expected in Lower Triassic or earlier times. If *Eunotosaurus* (Watson, 1914b) from the Middle Permian is a chelonian then common ancestry would be expected in Lower or Middle Permian times. A Permian origin would be more reasonable than an origin in early Upper Carboniferous times or even in the Lower Carboniferous as required by the theories of Watson and Romer and the earlier theories of von Huene.

As mentioned above, the most primitive known chelonians are too advanced to be direct ichthyosaur ancestors. In some ways chelonians show advances in regions which remain primitive in ichthyosaurs, e.g. in the loss of the pineal opening and the swinging down of the paroccipital processes to the almost horizontal position. The ichthyosaurs also show advances in regions which remain primitive in chelonians, e.g. the acquisition of the temporal opening.

The most important of such advances is the loss of the otic notch and the presence of the contact between the distal end of the stapes and the quadrate. Chelonians differ from the ichthyosaurs in that the simple rod-like stapes inserts into a tympanic membrane spanning an otic notch.

The anatomy of the ichthyosaurian ear has led Watson and Romer and others to classify ichthyosaurs with " theropsids " (p. 356).

When examined in detail the primitive ichthyosaur ear is seen to differ from the ears of pelycosaurs and *Captorhinus* in that the dorsal process, stapedial foramen and distal cartilaginous extension of the stapes are absent. Primitively the ichthyosaurian stapes was medium to lightly built and not heavy as in the later *Ophthalmosaurus* (p. 343).

Parrington (1955), from a consideration of the homologies of the external auditory meatal groove in therapsids and the stapes in *Captorhinus*, has shown that, there were both quadrate and tympanic processes in Captorhinomorpha and Pelycosauria. The tympanum was postquadrate in these groups. In ichthyosaurs the whole stapes from the proximal contacts to the distal contact with the quadrate are ossified (p. 343). There are no roughenings along its

length for the origin of a cartilaginous tympanic process. There was therefore no separate tympanic process, which indicates that there was no post-quadrate tympanum in ichthyosaurs.

Conditions exactly similar to the ichthyosaurian are seen in modern lizards (Smith, 1938) where, in agamids and iguanids, complete morphological series are seen between forms with dorsal, quadrate and tympanic processes and forms with the single rod-like stapes abutting distally against the quadrate. These series suggest that the tympanic and quadrate processes coalesce to become one, i.e. the single distal stapedial process is not homologous with the quadrate process of pelycosaurs and captorhinomorphs.

Among Permian forms the ichthyosaurian condition of the stapes is seen in *Nyctiphruretus* where a single rod-like stapes passes from the fenestra ovalis to abut against the quadrate.

Thus, the ichthyosaurian ear, though superficially similar to the pelycosaur ear, is fundamentally different and is similar to the ears of certain " diadectomorphs " and lizards.

If ichthyosaurs and chelonians are more closely related to each other than to any other group their common ancestor would be expected to possess the advanced characters held in common by the two groups, together with any primitive tetrapod characters found separately in either group. Principal among the latter are the chelonian ear with its otic notch, tympanic membrane and stapes inserted into the membrane, and the ichthyosaurian dorsolaterally directed paroccipital process, open palate and posteriorly placed jaw condyle with sloping quadrate, in addition to the remainder mentioned on page 357.

Palaeontological opinion has favoured a diadectomorphan ancestry for the Chelonia. Gregory (1946) has derived them from pareiasaurs, Olsen (1947) from diadectids and Romer (1956) from Diadectomorpha.

The largest number of characters of the hypothetical ichthyosaur–chelonian ancestral stock is found in primitive procolophonids. Many of these are primitive characters such as the anapsid skull roof, the possbile presence of palatal teeth, the marginal conical teeth, the presence of interpterygoid vacuities (*Nycteroleter* and *Owenetta*), the dorso-laterally directed paroccipital process (nyctiphruretids) and the reduced ossification in the ear region (*Procolophon* (Watson, 1914a)). A number of advances made in common by ichthyosaurs and chelonians is also present in some procolophonids. In *Owenetta*, for example, the meeting of the postfrontal with the supratemporal is a similarity to the ichthyosaurs and chelonians. There are also large posttemporal fossae and cranioquadrate passages and posterior exposure of the quadrate in nyctiphruretids while in *Nyctiphruretus* the stapedial foramen and processes are absent.

In addition to the common possession of these characters the two stocks show a tendency to work out common trends. This includes a tendency for some lines to take up an aquatic mode of life (Watson, 1957, p. 385 on aquatic procolophonids); the interpterygoid vacuities become smaller as the palate closes in procolophonids and chelonians; and the jaw articulation moves anteriorly in members of the three stocks. In support of the latter point, Efremov (1940b) mentions, but does not name, a nyctiphruretid in which the jaw

articulation is more posteriorly place than in *Nycteroleter ineptus* where the jaw condyle is in approximately the same transverse vertical plane as the occipital condyle. This is, therefore, similar to the primitive ichthyosaur condition as seen in *Cymbospondylus*. There is also a tendency for the simple rod-like stapes to move onto the quadrate in ichthyosaurs and nyctiphruretids but not in chelonians and *Procolophon*.

In view of this evidence there is reason to think that the ichthyosaur-chelonian stock might have had common ancestry with procolophonids in Lower Permian or earlier times.

Efremov further believes that procolophonids and pareiassaurs had a common ancestry " perhaps not earlier than Zone 1 of the Russian Permian " (Watson, 1954 p. 132). If this is so, the common ancestor must have been a very primitive tetrapod, combining the primitive features of both groups, such as the primitive frontal and nasal regions of pareiasaurs with the primitive occipital region, open palate and posteriorly placed jaw condyles of primitive procolophonids.

This stock would be much more primitive than *Diadectes* or *Bolosaurus*, both of which have a number of specializations and this would suggest that the line leading to the common ancestral stock of procolophonids and pareiasaurs separated from the line leading to the diadectids prior to the occurrence of the first diadectids, i.e. prior to Conemaugh times in the Pennsylvanian (Watson, 1954 ; 1957 for stratigraphical occurrences). If the separate stock leading to pareiasaurs and procolophonids was present at the base of the Russian Permian it would be an even more ideal ancestor for the ichthyosaur-chelonian stock than the primitive procolophonids.

Before the ichthyosaurian-chelonian stock is related with any certainty to the ancestral procolophonids or to the procolophonid-pareiasaurian stock two regions of the skull must be discussed. The first is the palato-quadrate cartilage together with the basipterygoid articulation and the second is the temporal region.

The ichthyosaur palate is more primitive than those of most procolophonids, pareiasaurs or chelonians in that interpterygoid vacuities are present. Unfortunately, the internals of the palate cannot be compared for in pareiasaurs and late chelonians the palates are too specialised while in procolophonids and *Triassochelys* the internals of the palate are unknown.

The palate of ichthyosaurs is described above (pp. 351–353) and there is nothing to prevent the ichthyosaur condition from giving rise to the more specialized conditions of pareiasaurs, procolophonids and chelonia. The ichthyosaurian condition is not, however, the primitive tetrapod one but resembles the condition in millerosaurs. It is thus necessary to examine the evolutionary position of the millerosaurs which is unfortunately in dispute. Watson (1957) believes that they share common ancestry with the procolophonids, a theory which, if true, would support the derivation of ichthyosaurs and chelonians from a procolophonid stock. Parrington (1958) disagrees with Watson and suggests that millerosaurs originated among or close to Captorhinomorpha. If this theory is the true one the ichthyosaurs share a large number of primitive and advanced characters with the procolophonids

and have palatal advances in common with captorhinomorph derivatives. In this context it is interesting to note that the similarities between millerosaurs and ichthyosaurs in the palatal region are points upon which millerosaurs differ from Captorhinomorpha.

In reaching his conclusion Parrington compared a number of features of millerosaurs with homologous regions in captorhinomorphs and pelycosaurs on the one hand and with similar regions in diadectomorphs on the other hand.

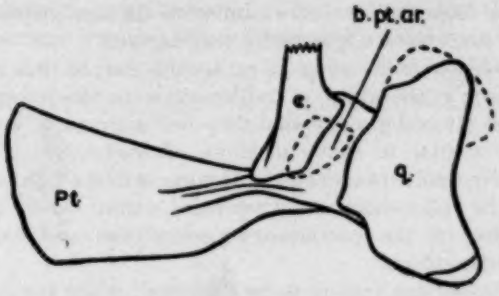

Fig. 16—Lateral view of the palatoquadrate region in *Ichthyosaurus* showing the relative positions of the epipterygoid and basipterygoid articulation (Leicester Museum 100'1949/111) × ¼ approx.

Many of the features were of little significance on their own, as he admits, but taken together they showed a strong likelihood that millerosaurs were of captorhinomorph rather than diadectomorph origin. The similarities to captorhinomorphs included the presence of an elongate skull, the presence of a prefrontal boss or swelling, the high position of the jugal posteriorly, the slender form of the postorbital and its posterior extension, the presence of a slender or greatly reduced supratemporal, the presence of synapsid fenestration, the presence of conical pointed teeth, the enlargement of anterior maxilliary teeth, the presence of the tabular and postparietal largely or entirely on the occiput, the breadth of the supraoccipital and the modified parasphenoid-basisphenoid complex.

These features were examined in six millerosaurian genera, the majority of which possess most of them. Four captorhinomorphs and primitive pelycosaurs also possessed most of them while the features only occurred infrequently among procolophonids, pareiasaurs and diadectids, which Parrington places together as one group.

Parrington also examined four dissimilarities between millerosaurs and the captorhinomorph-pelycosaur group and showed that the millerosaur condition could be derived from the captorhinomorph-pelycosaur condition.

There are two main criticisms of Parrington's theory. Firstly, comparisons made between only the primitive members of the three groups show a less definite relationship. Millerosaurs bear more resemblance to captorhinids than to *Limnoscelis*. Secondly, it is likely that *Mesenosaurus* had a dorso-laterally directed paroccipital process. This follows from Watson's (1957) statement that " the end of the paroccipital process must have had a contact

with the freely projecting end of the supratemporal . . ." in *Mesenosaurus*. This is supported by the slight variations in the direction of the paroccipital processes of *Millerosaurus* and *Milleretta* (Watson, 1957). Since the paroccipital processes of all captorhinomorphs and pelycosaurs are either horizontally or ventrolaterally directed, the millerosaurs must have been derived from a forerunner of the known captorhinomorphs and pelycosaurs. This at once places the common ancestry of millerosaurs and the captorhinomorph-pelycosaur stock in the Upper Carboniferous. If *Limnoscelis* is morphologically antecedent to the captorhinids then a number of the similarities of millerosaurs to captorhinids must have appeared by convergence.

The relationship of millerosaurs to captorhinomorphs thus rests upon slight evidence. Watson's allocation of millerosaurs to diadectomorph ancestry also rests upon slight evidence, i.e. that they are "sauropsid" and have parietals which are very similar in shape to those of *Nycteroleter*. This leaves the similarities between millerosaurs and ichthyosaurs in the palatal region difficult to evaluate. The millerosaurs could represent a third line of descent from the common ancestor of the pareiasaur-procolophonid and captorhinomorph-pelycosaur ancestral stocks.

The second important feature to be discussed, is the temporal region (Fig. 17). Watson (1954) has given an account of the temporal region in primitive "theropsids" and "sauropsids" and has described their common possession of a parietal lappet filling the space occupied, in anthracosaurs, by the intertemporal. Parrington (1958) also believes that the parietal lappet was a feature of the common ancestor of "diadectomorphs" and captorhinomorphs and shows it in his hypothetical protoamnicte.

He also shows a labyrinthodont condition in which the space occupied by the intertemporal is closed by the meeting of the supratemporal with the postfrontal. This latter condition is universally present in ichthyosaurs and is also present in *Triassochelys*, *Rhipaeosaurus* (Chudinov, 1955) and the procolophonid *Owenetta*. The remainder of the procolophonids possess a parietal lappet.

The presence of the ichthyosaurian condition in *Triassochelys* indicates that the common ancestral stock also possessed the condition. The ichthyosaur chelonian stock was therefore derived from pre-procolophonids with *either* an intertemporal *or* a parietal lappet separating the supratemporal from the postfrontal. The presence of the postfrontal meeting the supratemporal in *Owenetta* lends some support for the former alternative because *Owenetta* is looked upon as a primitive procolophonid (Romer, 1956). Since later procolophonids, which have reached more advanced stages in the typical procolophonid trends (e.g. *Procolophon*) do not acquire the *Owenetta* condition, that condition cannot be ascribed to any particular procolophonid adaptation. This leads to the conclusion that the intertemporal was present in the immediate ancestors of the procolophonids.

The nyctiphruretid *Nyctiboetus* may also support this theory for in this skull there are three bones between the postfrontal and the back of the skull. These have been labelled postorbital, supratemporal and tabular by Chudinov (1955) but the most anterior bone has little, if any, part in the orbital boundary and

is more dorsally placed than postorbitals in most procolophonids. If this bone is not a postorbital it can only be an intertemporal. If this is so it may well be that the bone labelled " st " in *Rhipaeosaurus* is an intertemporal also (Chudinov, 1955).

This conclusion would agree very well with the separate derivation of the procolophonid-pareiasaur stock from forerunners of known diadectids. If diadectids are more closely related to *Seymouria* than to anything else (Olson, 1947 ; Watson, 1954), the common ancestor of all the " Diadectomorpha " must have possessed an intertemporal. The problem then becomes that of deciding at what time between Upper Carboniferous times and *Deuterosaurus-*

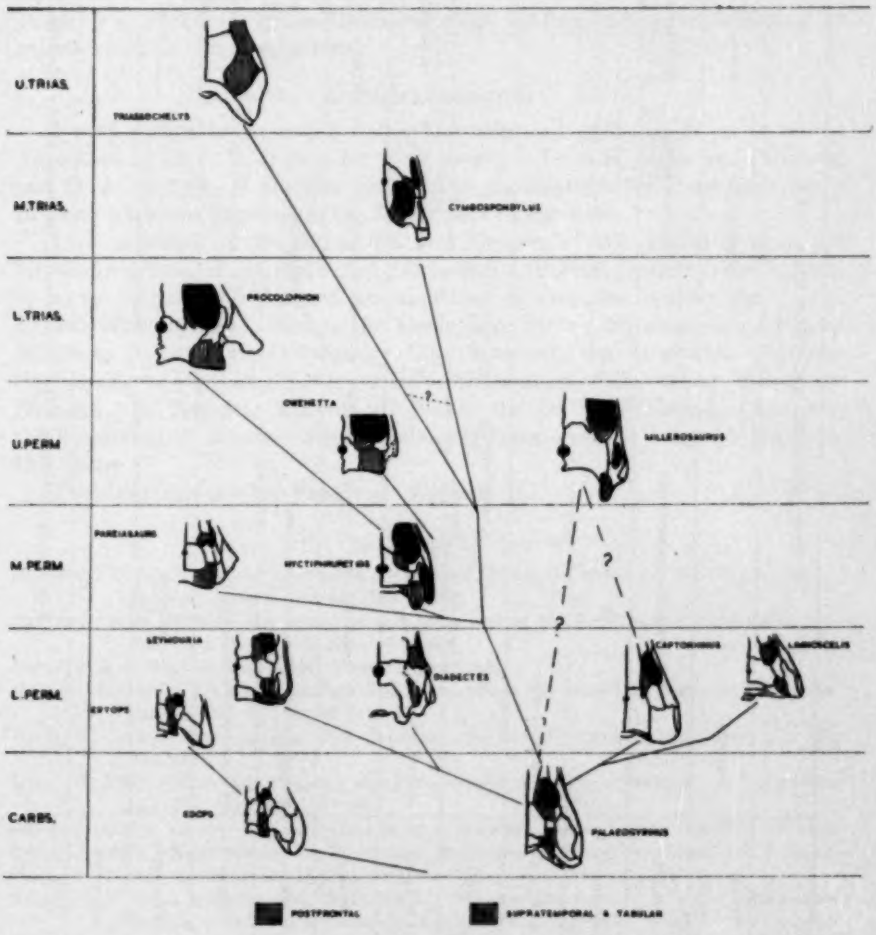

Fig. 17—The temporal region in primitive tetropods.

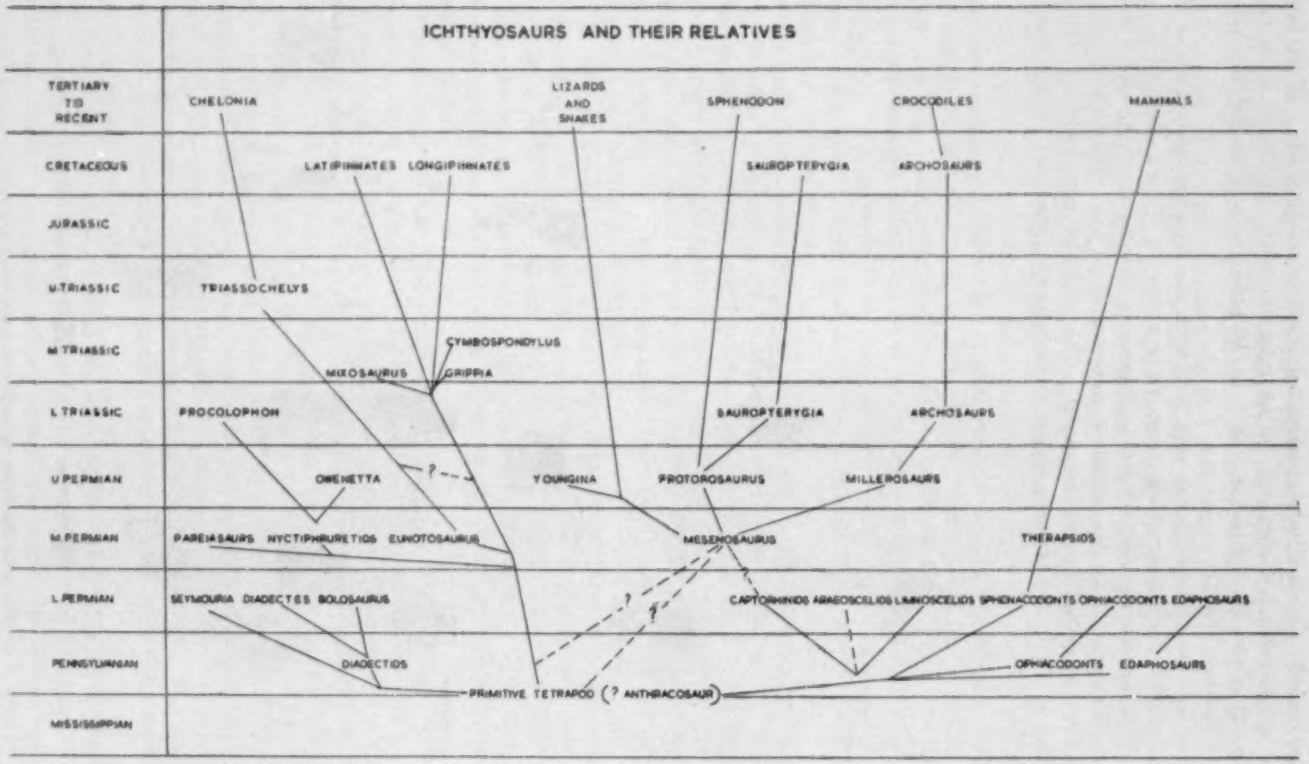

Fig. 18—Evolutionary " tree " of the ichthyosaurs and their relatives.

times the intertemporal was lost. If primitive procolophonids possessed it, and if Efremov is right in thinking that procolophonids and pareiasaurs had common ancestry in *Deuterosaurus*-times, then the intertemporal must have been present in *Deuterosaurus*-times at least.

From this evidence and reasoning it follows that the ichthyosaur-chelonian stock could not have separated from the pareiasaur-procolophonid stock earlier than a little time previous to the deposition of Zone 1 of the Russian Permian. From the first occurrence of the ichthyosaurs, which were already differentiated into latipinnate and longipinnate groups, the ichthyosaurian-chelonian stock could not have separated much later than the close of the Permian.

Whatever the ultimate derivation of the ichthyosaurs, the ichthyosaur-chelonian relationship is well founded and the common ancestor of the two groups was probably derived from the stock leading to procolophonids or to procolophonids and pareiasaurs.

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ABBREVIATIONS OF NAMES OF MUSEUMS

B.M.N.H.	British Museum (Natural History), London.
H.M.S.M.	Geological Survey Museum, London.
L.C.M.	Leicester City Museum.
M.U.M.	Manchester University Museum.
O.U.M.	Oxford University Museum.
P.M.	Peterborough Museum.
S.M.	Sedgwick Museum, Cambridge.

KEY TO REFERENCE LETTERING TO FIGURES

b.	Brain	n.c.	Nasal capsule
b.o.	Basioccipital	o.ca.	Otic capsule
b.pt.ar.	Basipterygoid articulation	op.	Opisthotic
b.pt.pr.	Basipterygoid process	p.	Parietal
b.sph.	Basisphenoid	Pmx.	Premaxilla
cart.	Cartilage	pof.	Postfrontal
e.	Epipterygoid	pr.	Prootic
e.n.o.	External nasal opening	pr.f.	Prefrontal
ex.	Exoccipital	Pt.	Pterygoid
f.	Frontal	Q.	Quadrate
fen.o.	Fenestra ovalis	q.	Quadrate
hyp.	Hypophyseal vein	Sm.	Septomaxilla
i.n.o.	Internal nasal opening	s.o.	Supraoccipital
Ju.	Jugal	st.	Supratemporal
L.	Lachrymal	sta.	Stapes.
l.h.v.	Lateral head vein	vn.o.	Vomeronasal organ
Mx.	Maxilla	IX	Foramen for glossopharyngeal nerve
N.	Nasal		
na.	Nasal	XII	Foremen for twelfth nerve

THE LARVAL STAGES OF *ACASTA SPONGITES* AND *PYRGOMA ANGLICUM* (CIRRIPEDIA)

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(With 11 figures in the text)

Descriptions based on reared larvae are given of the six naupliar and one cyprid stage of *Acasta spongites* Darwin and *Pyrgoma anglicum* Leach. In plankton from British coastal waters, confusion is most likely to occur between these nauplii and those of *Balanus perforatus* but they can be distinguished at all stages by the body proportions. The nauplii of *Acasta* are more slender and those of *Pyrgoma* more rounded than those of *B. perforatus*. Those of *Acasta* are also distinguished by having marginal spines. Other features are distinctive at certain stages. The seasons of occurrence in the plankton of other Balanidae from British waters are briefly reviewed.

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INTRODUCTION

Descriptions of the larval stages of cirripedes have revealed that it is simple to assign a nauplius to its correct family, but much more difficult to distinguish the genus or species. In practice, however, the number of species likely to occur in a given plankton sample is limited by their geographical distribution and times of breeding and it is therefore not necessary to provide a comprehensive key based on morphological differences alone.

The larvae of *Acasta spongites* Darwin and *Pyrgoma anglicum* Leach have not been described previously. Both these species have their centres of distribution towards the tropics and in Europe extend as far north as the western English Channel and southern Irish Sea. Both are essentially sublittoral, although *A. spongites* can also be collected intertidally at extreme low water. The following account aims simply at distinguishing their larvae from those of other Balanidae likely to occur with them in British coastal waters.

Descriptions of the feeding and swimming mechanisms of barnacle nauplii have been given by Lochhead (1936), Norris & Crisp (1953) and Gauld (1959).

MATERIAL AND METHODS

With exceptions mentioned later, all the descriptions in this account are of larvae reared in the laboratory. Adult barnacles were collected by diving at Martin's Haven and off Skomer, Pembrokeshire, to rocks below the *Laminaria* zone. *Pyrgoma anglicum* occurs only on cup corals, *Caryophyllia smithi* Stokes, which were knocked off the rocks with a hammer and chisel. *Acasta spongites*, which is particularly abundant off Skomer, lives embedded in the sponge *Dysidea* (=*Spongelia*) *fragilis* (Montagu) and this could be collected easily by hand. The collections were transported in breffits of clean sea-water back to Swansea, where the corals with their attached barnacles were kept alive for several months. *Dysidea* collected as above, however, is not readily kept alive in the laboratory and it was found to be advisable to dissect out the *Acasta* and transfer them to clean sea-water as soon as possible. Liberations of nauplii usually occurred soon after collection and further liberations occurred at intervals for some days afterwards. No effort was made to feed the adult barnacles or otherwise encourage breeding to continue in the laboratory.

Nauplii were pipetted off and washed by allowing them to swim through pasteurized sea-water. They were cultured as previously described (Moyse, 1960) on a diet of *Skeletonema costatum* (Plymouth Collection No. 106). Within a week cyprids were present, the laboratory temperature being generally about 20°C. Samples of the stirred cultures were poured daily through fine plankton silk and the larvae retained on the silk were fixed in 5 per cent formaldehyde in sea-water. This yielded abundant whole nauplii and exuviae of each stage. The mortality appeared to be low and large numbers of cyprids were produced. Some cyprids of *Pyrgoma* settled on cup corals which were offered to them.

Drawings were made using a squared eyepiece and graph paper, some of the details of setae and setulation being worked out from exuviae using phase contrast. The best way of holding the larvae for measurement was to use a "Perspex" slide having V-shaped grooves of various depths up to about 0·5 mm. made with a circular saw set at 45°, but the grooves had to be smooth sided to avoid scattering of the light. The larvae were aligned in the grooves with dissecting needles and measured using an eyepiece micrometer.

Measurements of "overall length" were made from the middle of the anterior border of the body to the tip of the caudal spine. This is the measurement used by previous workers and is probably the most useful for comparative purposes. It is not necessarily the maximum length however, for in *Acasta* the frontal horns reach forward of the body and in stage V and VI of *Pyrgoma* the distance to the tips of the caudal furca is often greater than to the tip of the caudal spine. Carapace length was measured to the posterior edge between the carapace spines and carapace breadth was taken as the greatest breadth behind the frontal horns. It is not possible to measure accurately the length of the frontal horns since these merge gradually into the body, so the most

useful measurement of them is the distance between their distal tips. Spines were measured on their shorter sides where there was usually an acute angle at the point of origin.

Setation formulae and setation tables were worked out according to the systems of Bassindale (1936), and Jones & Crisp (1954).

VALIDITY OF REARED MATERIAL, WITH SOME NOTES ON ECDYSIS

Series of developmental stages based on larvae reared in the laboratory have been described by various authors (notably Bassindale, 1936, Ishida & Yasugi, 1937 ; Hudinaga & Kasahara, 1941 ; Sandison, 1954 ; Costlow & Bookhout, 1957, 1959). It has been suggested, however, that the possibility of abnormalities arising in such material reduces its value for taxonomic purposes (Norris & Crisp, 1953 ; Barnes, 1959).

In the present study various structural irregularities have been observed but these have always been obvious, because they occurred in a small minority of larvae and because of the nature of the deformity. They fall into two categories, the first being associated with partial failure of ecdysis. In normal ecdysis the old cuticle splits along the anterior edges of the carapace, the anterior half of the carapace emerges with its frontal horns and stylets and the limbs are rapidly pulled free. The posterior edge of the carapace and the caudal spine and abdominal process are last to appear. The large, posteriorly directed spines of the abdominal process, present in all stages except the first, are possibly important in the final stage of ecdysis, helping to push off the cuticle of the previous stage. The six pairs of spines in stage VI are probably necessary to slough off the stage V cuticle from the greatly enlarging abdominal process.

In some cultures it occasionally happened that the final stage of ecdysis was not successfully completed, so that a nauplius identifiable from its anterior end and limbs as a particular stage still had the caudal spine, abdominal process, etc., of the previous stage. The partly sloughed exuvium could usually be seen, although some of it might get detached. This has been observed most often at moults II/III and III/IV and might be the explanation of the " developmental intermediates " of *Balanus perforatus* Brugière between stages I and II described by Norris & Crisp (1953). Such intermediates are not to be confused with the different degrees of development of certain characters at stages V and VI (see below) to which condition the term " developmental intermediate " has also been applied (Norris, Jones, Lovegrove & Crisp, 1951).

In the second category are occasional deformities perhaps resulting from encounter with the sides of culture vessels. These affect in particular the caudal spine and abdominal process which may be bent or more seriously malformed.

Some hundreds of nauplii were examined from plankton hauls taken off Martin's Haven, Pembrokeshire, in August 1959, but only a few stage II nauplii of *Acasta* and *Pyrgoma* were found amongst many larvae of *Balanus perforatus*, *Cthamalus stellatus* (Poli) and *Verruca stroemia* (O. F. Müller). It was therefore impossible to compare reared and wild larvae at later stages, except by rearing

TABLE 1. Measurements of length and breadth of larvae of *Acasta spongites* and *Pyrgoma*

		Balanus perforatus (From Norris & Crisp, 1953)			*Balanus perforatus* (From reared specimens)			*Balanus improvisus* (From Jones & Crisp, 1954)		
Stage		Mean	S.D.	No. in sample	Mean	S.D.	No. in sample	Mean	S.D.	No. in sample
I	Overall Length	279	± 8·3	6	284	±15·5	10	195	±19·7	10
	Carapace Breadth	131	± 6·0		132	± 7·9		111	± 8·4	
II	Overall Length	429	±12·9	6	437	±16·8	10	323	±16·6	10
	Carapace Breadth	175	± 4·4		173	± 4·4		147	± 8·8	
III	Overall Length	493	±18·3	6	493	±15·1	10	367	± 8·4	3
	Carapace Breadth	234	± 9·9		226	± 8·0		183	± 7·0	
IV	Overall Length	565	±31·8		580	±24·8		402	±15·4	
	Carapace Breadth	290	±10·7	6	277	±14·6	10	230	±19·6	10
	Carapace Length	338	±17·1		343	±13·0		267	±21·0	
V	Overall Length	716	±41·0		704	±21·0		496	±22·9	
	Carapace Breadth	377	±32·2	6	353	±32·0	10	281	±27·8	12
	Carapace Length	451	±23·5		439	±19·3		354	±17·5	
VI	Overall Length	860	±31·9		818	±24·9		624	±30·2	
	Carapace Breadth	481	±33·6	6	442	±24·1	10	370	±14·0	8
	Carapace Length	583	±28·9		562	±19·7		465	±16·3	
	Cyprid Length	698	±53·2	6	682	±43·1	8	523	±12·3	8
	Breadth (dorso-ventral)	295	±21·0		305	±38·7		235	±10·3	

anglicum—with those of other balanid species which could occur with them in British waters

Acasta spongites (From reared specimens)				*Pyrgoma anglicum* (From reared specimens)				*Elminius modestus* (From Knight-Jones & Waugh 1949)			
Mean	S.D.	Min. Max.	No. in sample	Mean	S.D.	Min. Max.	No. in sample	Mean	Min. Max.		No. in sample
309	± 8·2	293–320	10	300	±15·2	280–321	10	?	240–260		25
145	± 5·2	134–152		165	± 9·2	154–184		125	110–140		
497	± 7·0	474–513	10	448	±13·7	438–471	10	?	360–430		39
176	± 4·9	168–183		217	± 7·5	190–225		161	150–170		
546	± 9·6	523–560	10	498	±13·8	488–507	10	?	350–430		41
203	± 6·9	192–214		266	± 8·3	254–273		191	180–200		
595	±21·4	572–638		580	±25·7	544–610		?	390–500		
250	± 6·4	240–261	11	329	±15·9	310–362	10	237	220–250		48
338	±10·6	316–348		371	±16·4	340–390		?	290–350		
698	±15·5	672–723		696	±23·0	648–720		?	450–570		
306	±11·6	289–319	10	423	±13·4	399–456	10	298	260–310		48
406	± 8·7	400–418		489	±18·1	450–518		?	340–440		
803	±16·8	772–831		817	±28·7	780–864		?	480–710		
376	± 6·7	368–387	12	501	±15·2	480–523	10	359	330–410		44
491	±10·7	474–512		594	±17·0	568–630		?	420–550		
557	±19·4	524–592	10	695	±24·0	650–734	10	?	540–560		?
289	±16·5	270–328		322	± 9·2	310–340		?	?	?	?

larvae of *B. perforatus* and comparing these with wild larvae and published measurements.

The reared and wild larvae thus compared were found to be very similar in general appearance and in features of the type used as diagnostic for *Acasta* and *Pyrgoma*. Details of mean overall size for ten reared specimens compared with published figures (Norris & Crisp, 1953) for the same measurements are given in Table 1. There is a slight tendency for reared larvae to be smaller than the wild ones, especially in breadth measurements, but the differences are within the normal range of variation.

To guard against the dangers of using reared material, the following descriptions are based upon larvae from cultures in which there was negligible mortality at all stages. The characters suggested for identification are such as to be little affected by a slight departure from mean size.

THE SIX NAUPLIAR STAGES OF BALANIDAE

The present species, like Cirripedia previously studied, have six naupliar stages and one cyprid stage. They also possess in common with other Balanidae a characteristically trilobed labrum.

Enough genera and species of Balanidae have now been described to justify the provisional compilation of a table by which any balanid nauplius may be assigned to its correct stage whether or not its species is known. It is in fact necessary to identify the stage if size is to be used in species identification. Of particular significance is the number of spines developed on the abdominal process (Fig. 1 ; Table 2) which can be referred to using the notation devised by Norris & Crisp (1953) for *Balanus perforatus*. In this the spines are numbered according to the order of their appearance during the development and they are not numbered in a regular order in later larvae. It should also be noted that some of the spines described as abdominal lie opposite the swimming legs of the future cyprid, which are usually referred to as thoracic limbs. It is doubtful, however, whether useful and well-established nomenclature should be changed to cure such slight anomalies.

TABLE 2. Distribution of abdominal spines in nauplii of Balanidae.

Stage	Series 1	Series 2	Series 3
I	None	None	None
II	1 Pair	None	None
III	1 Pair	None	None
IV	1 Pair	1 Pair 1 Median	None
V	1 Pair	1 or 2 Pairs, 0 or 1 Median	1 Pair
VI	1 Pair	6 Pairs	1 Pair

Order of Appearance :

Series 1. Most posterior and largest pair. Placed ventro-laterally on abdominal process.

Series 2. Anterior to series 1 and 3 and in ventral or ventro-lateral position.

Series 3. Placed anteriorly to series 1 and on the dorso-lateral side of the abdominal process.

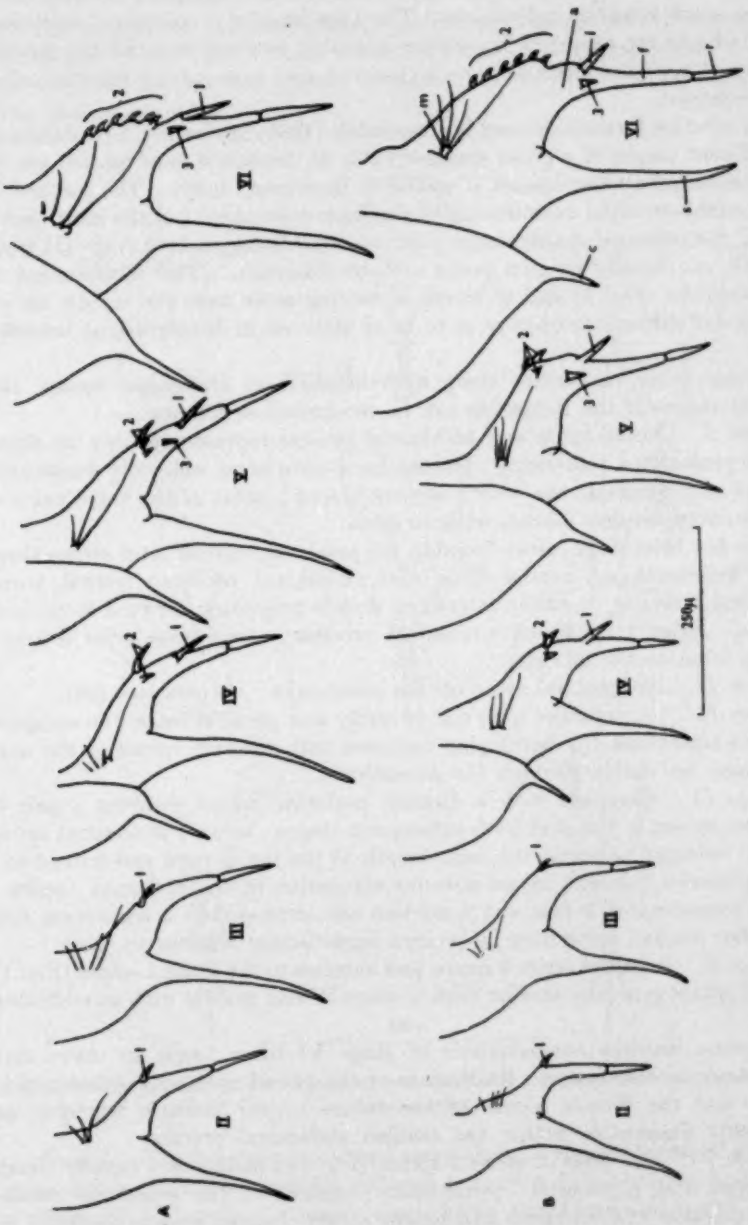

Fig. 1—Lateral views of posterior regions of stages II–VI of A, *Acasta spongites* and B, *Pyrgoma anglicum* showing a, abdominal process ; c, carapace spine ; f, stem of caudal furca ; m, maxillule ; r, ramus of caudal furca ; s, caudal spine.

Various additional small spines are present on parts of the abdominal process and caudal spine but these are of little use for taxonomy because they vary so much between individuals. The even smaller " spinules " scattered over the limbs are arranged in patterns probably characteristic for the species (see Figs. 4 & 5), but these have been observed with ease only on exuviae using phase contrast.

The setation formula devised by Bassindale (1936) can be used to distinguish the different stages of a given species (Table 3), though it is of limited use in distinguishing between species (Costlow & Bookhout, 1959). The method is not amenable to rapid examination of plankton collections, but the most useful feature, the presence of antennular preaxial setae distinguishing stage III from stage II, can usually be seen easily without dissection. The development of the maxillules (Fig. 1) and of tracts of feeding setae near the mouth are so gradual and difficult to observe as to be of little use in distinguishing between stages.

Listing other characters along with numbers of abdominal spines, the naupliar stages of the Balanidae can be recognized as follows :—

Stage I. Caudal spine and abdominal process represented only by short conical projections posteriorly ; frontal horns projecting obliquely backwards often closely alongside the body ; stylets absent ; setae of the three pairs of limbs without setules; labrum without setae.

This is a brief stage rarely found in the plankton. In all later stages there are a well-developed caudal spine and abdominal process ; frontal horns projecting laterally or antero-laterally ; stylets projecting forward from near the eye ; series 1 spines on abdominal process ; many setae with setules ; feeding setae on the labrum.

Stage II. No preaxial setae on the antennules ; no carapace fold.

Stage III. Antennules with one or rarely two preaxial setae ; no carapace fold but sometimes the developing carapace and carapace spines of the next stage may be visible through the exoskeleton.

Stage IV. Carapace with a distinct posterior border, bearing a pair of carapace spines in this and both subsequent stages ; series 1 abdominal spines (Fig. 1) enlarged to about the same length as the furcal rami and moved to a more posterior position by an anterior elongation of the abdomen ; series 2 spines consisting of a pair and a median one, arranged in a transverse row. A further median spine may be present immediately anterior to these.

Stage V. A pair of series 3 spines just anterior to the series 1 spines (Fig. 1); series 2 spines generally smaller than in stage IV and usually with an additional pair.

Various features characteristic of stage VI often begin to make their appearance in this instar. Rudiments of the paired eyes may sometimes be visible and the muscle blocks of the future cyprid thoracic segments are frequently discernible within the swollen abdominal process.

Stage VI. Six pairs of series 2 spines (Fig. 1) ; paired eyes usually clearly developed and pigmented ; penultimate segment of the antennule swollen in the position of the cyprid attachment sucker ; paired muscle blocks of the cyprid " thorax " clearly visible within the " abdomen ".

NAUPLIAR STAGES OF *ACASTA SPONGITES* Darwin

(Figs. 1, 2, 4 and 6 ; Tables 1 and 3)

The nauplii are characteristically slender with spines and processes longer than those of any other British balanid yet described. Small spines bordering the carapace are another unique feature. The labrum is square in outline. The stylets are long.

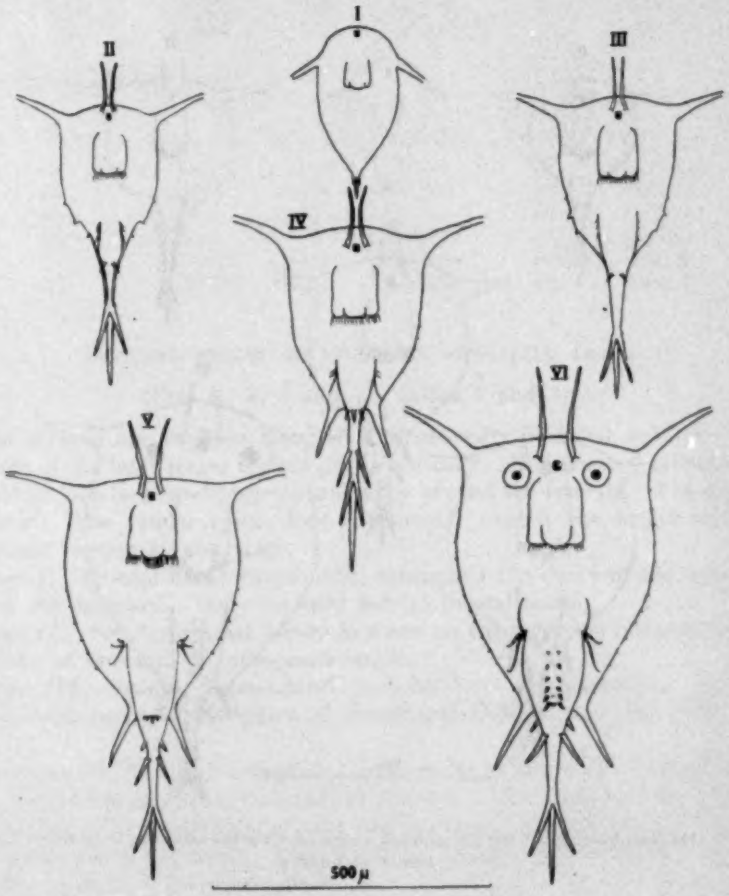

Fig. 2—Outline drawings of the six nauplior stages of *Acasta spongites* from which the limbs have been omitted.

Stage I. The frontal horns stand clear of the body, the sides of which are more or less parallel for a short distance immediately behind the horns.

Stage II. There is a postero-lateral spine and associated notch on each side of the body, similar to that occurring in *Balanus perforatus* which it has

been suggested may mark the posterior border of the carapace. There is usually a smaller spine anterior to the main one and less often another one posterior to it.

Both antennae and mandibles in this and all further stages bear a row of sessile preaxial setules (see Fig. 4).

Stage III. The notch on the posterior edge of the carapace is less marked than in Stage II.

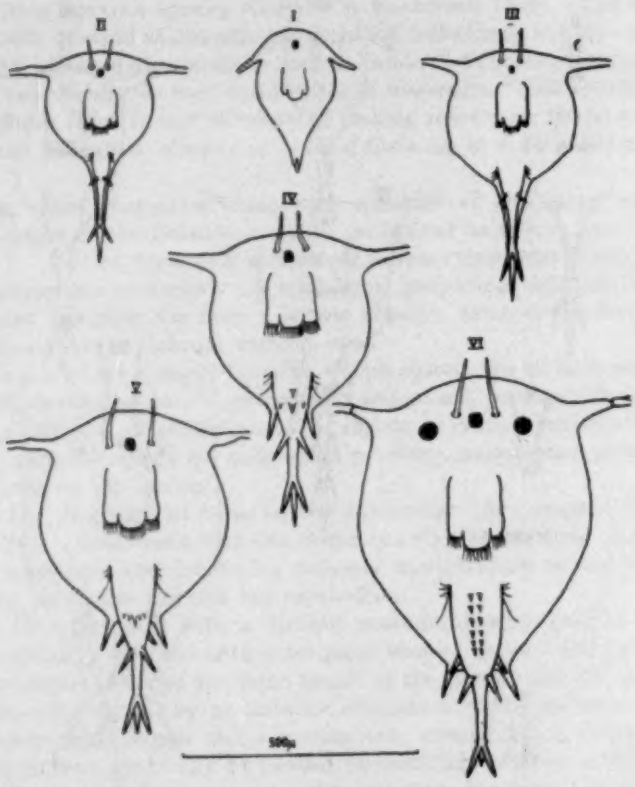

Fig. 3—Outline drawings of the six naupliar stages of *Pyrgoma anglicum* from which the limbs have been omitted.

Stage IV. The tiny spines around the edge of the carapace disappear from sight in slightly oblique viewing. Series 1 spines are greatly developed so that in some specimens their tips are posterior to the base of the furca.

Stage V. The series 1 spines have moved forward again, lengthening the furcal stem. The rami are also longer.

Stage VI. The long spines and bespectacled paired eyes make this nauplius unmistakable.

TABLE 3—Setation formula (Bassindale, 1936) for the naupliar stages of :

(a) *Acasta spongites*

Stage	Antennule	Antenna		Mandible	
		Exopodite	Endopodite	Exopodite	Endopodite
I	04211	014	03222 G	013	03222 G
II	04211	025	03222 G	013	03232 G
III	14211	025	03223 G	014	03333 G
IV	114211	026	04324 G	014	04333 G
V	11142111	036	04324 G	014	04343 G
VI	11142121	036	04324 G	014	04343 G

(b) *Pyrgoma anglicum*

Stage	Antennule	Antenna		Mandible	
		Exopodite	Endopodite	Exopodite	Endopodite
I	04211	014	03222 G	013	03222 G
II	04211	025	03222 G	013	03232 G
III	14211	025	03223 G	014	03333 G
IV	114211	027	04323 G	014	04343 G
V	11142111	037	04324 G	015	04343 G
VI	11142121	037	04324 G	015	04443 G

NAUPLIAR STAGES OF *PYRGOMA ANGLICUM* Leach

(Figs. 1, 3, 5 and 7 ; Tables 1 and 3)

The nauplii are medium sized with a generally rounded outline. The carapace of the later stages is deep dorso-ventrally. The trilobed labrum has the middle lobe large and projecting slightly beyond the laterals. The stylets are short. The caudal spine does not greatly exceed the length of the abdominal process at any stage.

Stage I. Frontal horns swept back, continuing the curve of the anterior edge of the carapace. Body rounded behind frontal horns.

Stage II. Sessile preaxial setules in a row on the antennae but not on the mandibles of this and all subsequent stages.

Stage III. Small postero-lateral protuberances are sometimes present (Fig. 3) reminiscent of the spines of *Acasta* and *Balanus perforatus*, but less sharp.

In stages IV, V, and VI there is a progressive swing of the frontal horns into a ventro-lateral rather than lateral position. The relative length of the caudal spine to the abdominal process decreases until in stage VI it is shorter than the abdominal process. This is partly brought about by an unusual increase in length of the furcal stem.

COMPARISON WITH ALLIED FORMS

In mixed plankton collections, a nauplius of the Balanidae may be distinguished by its trilobed labrum. In the Verrucidae and Chthamalidae the labrum is single lobed and the setae are downy with numerous setules. Also, a nauplius of *Chthamalus* is particularly short in relation to its breadth, whilst one of *Verruca* has a characteristically long and evenly tapering caudal region

(Bassindale, 1936). Nauplii of other families of Cirripedia show even less resemblance to the Balanidae.

In British waters nauplii of *Acasta* and *Pyrgoma* are likely to be found only in summer and autumn. There are only four other species of Balanidae, namely *Balanus spongicola* Brown, *B. perforatus*, *B. improvisus* Darwin and

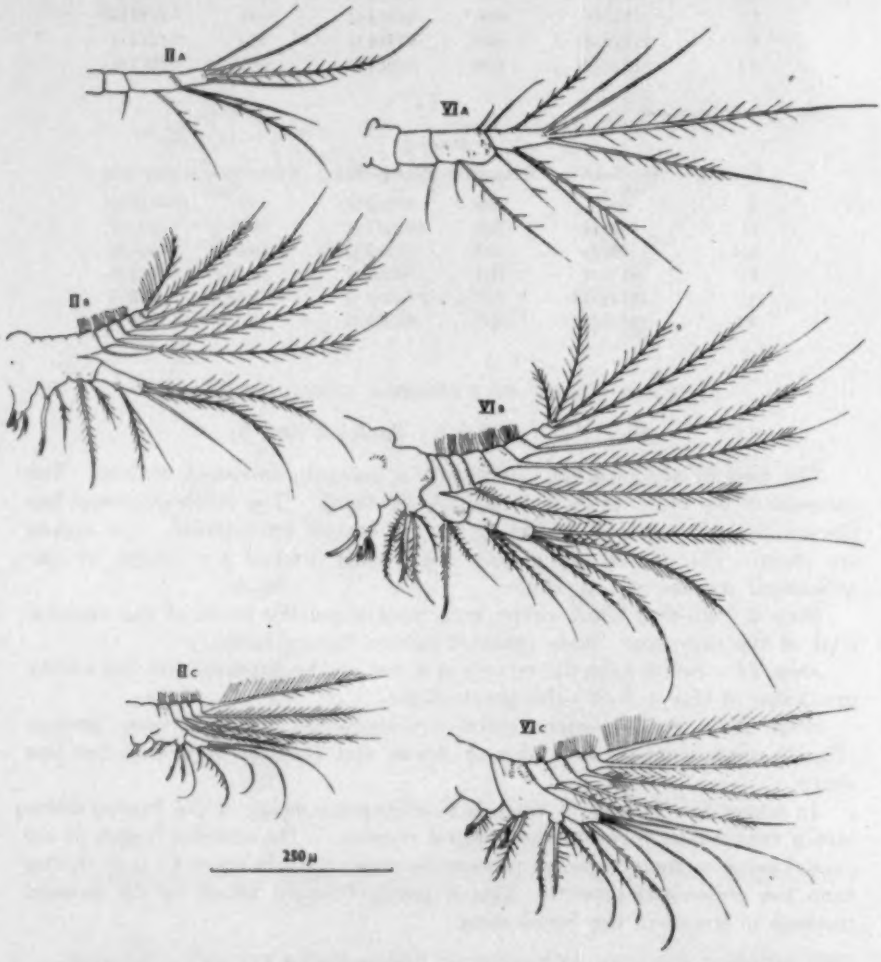

Fig. 4—The limbs of stage II and stage VI nauplii of *Acasta spongites*. A, antennule ; B, antenna ; C, mandible.

Elminius modestus Darwin, which would occur as nauplii at the same time (Table 4). Of these six species, nothing is known about the larvae of *B. spongicola* but the nauplii of *B. improvisus* and *E. modestus* may be separated

from the rest by their small size (Table 1) and by the fact that the labrum of *Elminius* has an elongated median lobe (Knight-Jones & Waugh, 1949).

Inspection of the measurements of the three species *A. spongites*, *P. anglicum* and *B. perforatus* shows that they cannot safely be separated on overall measurements alone, with the exception of the late stages of *Acasta*

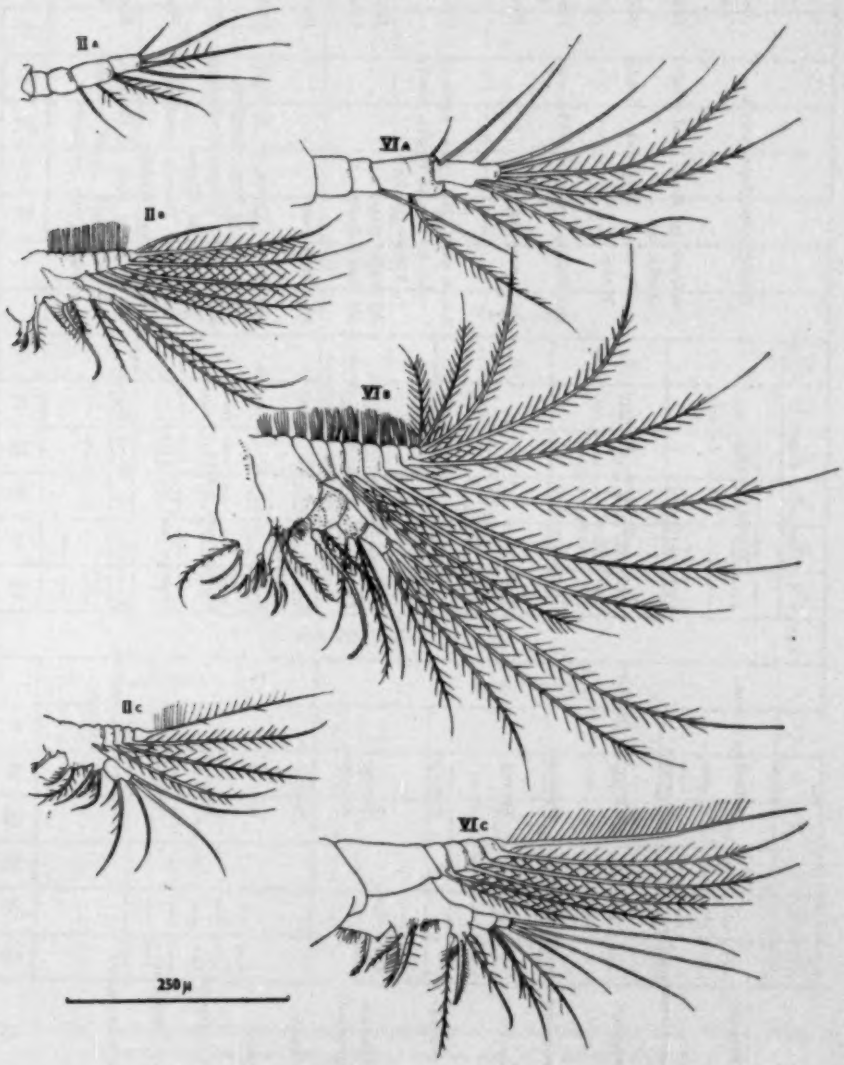

Fig. 5—The limbs of stage II and stage VI nauplii of *Pyrgoma anglicum*. A, antennule; B, antenna; C, mandible.

TABLE 4—Balanidae found in Britain.

Species	Breeding season	World distribution	British Isles distribution	Description of nauplii (most recent if more than one)
Balanus tintinnabulum	Summer	Circumtropical	Confined to ships and warm docks, etc.	Daniel, 1958
B. spongicola	Probably summer	East and West Atlantic, S. Africa Not in colder seas	South-west England, South Wales	Still undescribed
B. amphitrite var *denticulata*	Summer	Circumtropical	Artificially warmed docks, etc.	Costlow & Bookhout, 1958.
B. crenatus	Spring	Europe, N. America, West Indies, Cape of Good Hope Not in tropics	All round coast	Pyefinch, 1949
B. perforatus	Summer	Southern Europe and N. Africa	South-west but not Ireland (east to Isle of Wight, north to Pembrokeshire)	Crisp, 1953
B. balanoides	Spring	Europe, N. America, Arctic	All round coast	Bassindale, 1936
B. improvisus	Summer	Europe, N. America and west coasts of N. and S. America	All round coast	Crisp, 1954
B. balanus	Spring	Europe and N. America, Arctic	All round coast	Barnes & Costlow, 1961
B. hameri	Spring	Europe and N. America, Arctic	Northern. Down to N. Irish Sea, Isle of Man, Anglesey	Crisp (to be published)
Acasta spongites	Summer	Europe and Africa	South-west Britain (including South-west Wales)	Present account
Pyrgoma anglicum	Summer	Europe and W. Africa	South-west Britain (including South-west Wales)	Present account
Elminius modestus	All months, particularly summer	Australia, N. West Europe, etc.	Round large part of English, Welsh, and Irish coasts, spreading into Scotland	Knight-Jones & Waugh, 1949

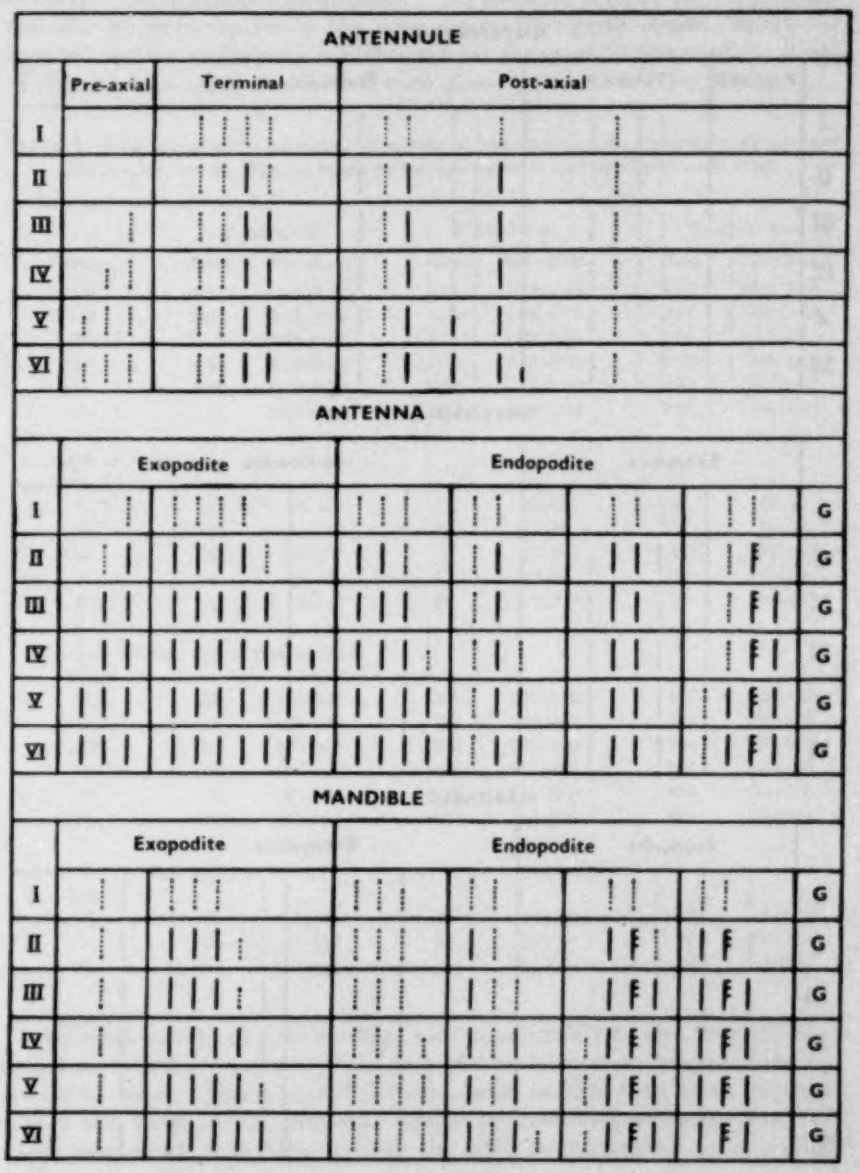

Fig. 6—The setation of *Acasta spongites* illustrated conventionally (Jones & Crisp 1954). Continuous line, seta with setules ; broken line, seta without setules.

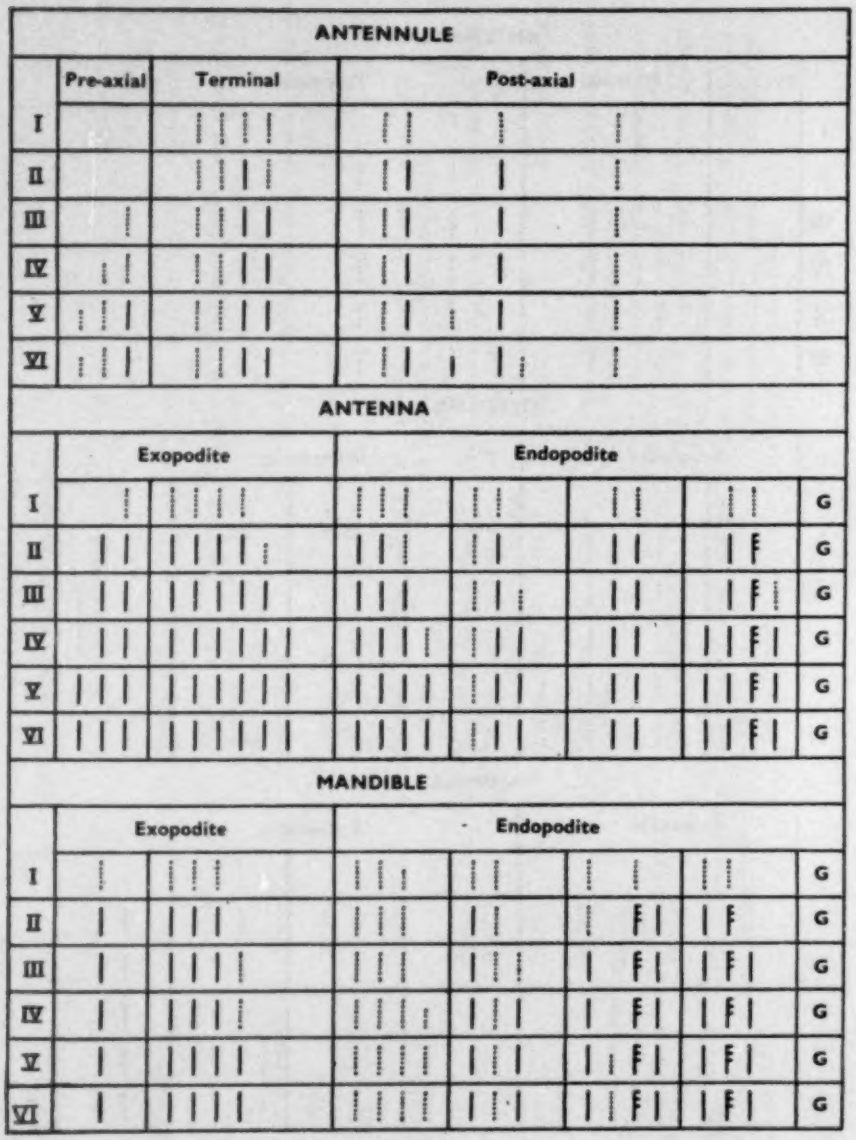

Fig. 7—The setation of *Pyrgoma anglicum* illustrated as in Fig. 6.

where the carapace breadth is distinctly smaller. Carapace shape however is readily distinguished with practice. The straight sides of the early stages and the bordering spines in the later stages distinguish *Acasta*, whilst the rounded outline of *Pyrgoma* is different from the graceful intermediate shape of *B. perforatus* (compared with a wine glass by Norris & Crisp, 1953).

TABLE 5—Some diagnostic dimensions of nauplii of *Acasta spongites*, *Pyrgoma anglicum* and *Balanus perforatus*. Figures based on ten specimens of each species at each stage.

	A. spongites		*P. anglicum*		*B. perforatus*	
Stage	Mean	Min.–Max.	Mean	Min.–Max.	Mean	Min.–Max.
Length/Breadth ratio						
I	2·12	2·02–2·37	1·81	1·73–1·93	2·07	1·90–2·16
II	2·80	2·72–2·98	1·96	1·88–2·02	2·47	2·32–2·54
III	2·70	2·58–2·82	1·88	1·82–1·96	2·37	2·28–2·49
IV	2·38	2·23–2·59	1·77	1·66–1·86	2·13	1·98–2·22
V	2·28	2·18–2·37	1·69	1·62–1·74	1·90	1·72–2·01
VI	2·14	2·00–2·24	1·64	1·56–1·72	1·85	1·76–1·96
Length of furcal rami						
II	84	78–90	62	58–68	57	48–60
III	81	68–96	53	48–60	51	46–59
IV	75	67–87	50	35–58	41	34–52
V	90	82–96	57	50–66	63	57–70
VI	91	82–107	66	58–73	77	67–86
Distance between tips of frontal horns						
I	230	218–240	261	230–285	177	165–190
II	364	348–379	382	362–402	303	280–320
III	369	330–396	390	361–400	324	310–335
IV	430	412–450	450	401–492	385	365–400
V	512	496–530	531	478–582	436	418–450
VI	557	534–580	589	565–625	501	470–534
Length of carapace spines						
IV	88	75– 96	69	65– 76	59	48–67
V	104	86–112	81	73– 90	61	52–71
VI	133	119–150	92	82–100	68	55–79

Although nauplii of a given stage vary considerably in size their proportions remain much the same. Thus ratio of overall length to carapace breadth is a useful figure. This is more than 2·0 in *Acasta* and less than 2·0 in *Pyrgoma* (Fig. 8 and Table 5). *B. perforatus* nauplii are intermediate but at Stage II where confusion is most likely to occur the ratios do not overlap so are a good guide to species.

Length, angle and form of spines and processes are at some stages diagnostic. *Acasta* is usually distinguished by its very long furcal rami and carapace spines (Fig. 9 and Table 5). The delicate, narrowly diverging furcal rami of *B. perforatus* contrast with the widely diverging furca of *Pyrgoma*.

The long frontal horns of *Acasta* project somewhat forward of the carapace. Although the figures of Norris & Crisp (1953) indicate that those of *B. perforatus* do so too, they in fact resemble those of *Pyrgoma* in not projecting forward so markedly. The distance between their distal tips is greater in *Pyrgoma* than in *B. perforatus* (Fig. 10 and Table 5), especially in the early stages where it is a useful diagnostic character.

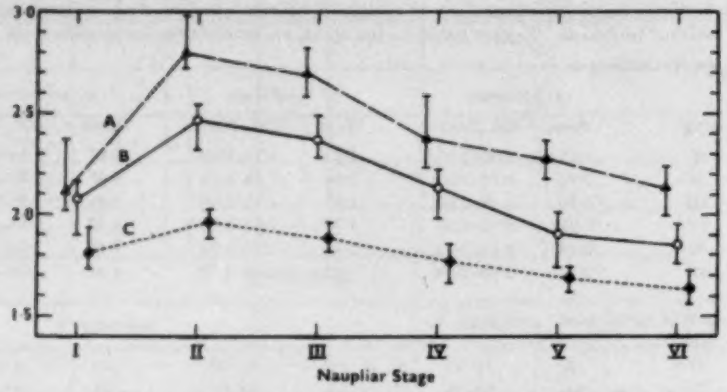

Fig. 8—Length/Breadth ratios of nauplii of A, *Acasta spongites*; B, *Balanus perforatus*; C, *Pyrgoma anglicum*. Means and extremes of ten specimens of each stage of each species.

KEY TO THE NAUPLII OF *ACASTA SPONGITES*, *PYRGOMA ANGLICUM* AND *BALANUS PERFORATUS*

It is first necessary to ascribe the specimen to the correct naupliar stage (see § 3).

Stage I.

(i) Distance between tips of frontal horns 200μ or less :—*B. perforatus*.
(ii) Distance between tips of frontal horns over 220μ
  (*a*) Ratio length to breadth 2 or more :—*A. spongites*.
  (*b*) Ratio length to breadth less than 1·95 :—*P. anglicum*.

Stage II.

(i) No small spines on postero-lateral margins of body ; frontal horns not projecting forward :—*P. anglicum*.
(ii) One to three small spines on postero-lateral margins of body.
  (*a*) Furcal rami over 75μ ; sides of body parallel ; frontal horns projecting somewhat forwards :—*A. spongites*.
  (*b*) Furcal rami under 70μ ; body widest half way along its length ; frontal horns project slightly forward but not in front of body :—*B. perforatus*.

Stage III.

(i) Frontal horns projecting well forward of anterior edge ; furcal rami over 68μ :—*A. spongites*.

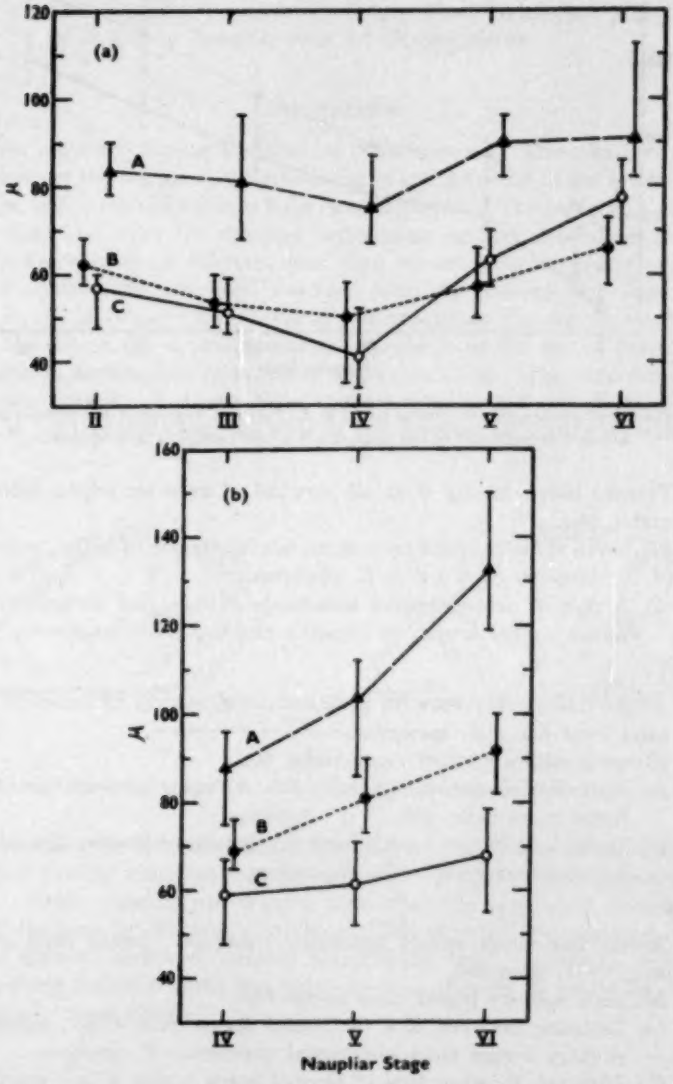

Fig. 9—a. Length of furcal rami at each stage for the three species: A, *Acasta spongites*; B, *Pyrgoma anglicum*; C, *Balanus perforatus*. b. Length of carapace spines of stages IV, V, and VI for the same species as in a. Means and extremes of ten individuals of each stage of each species.

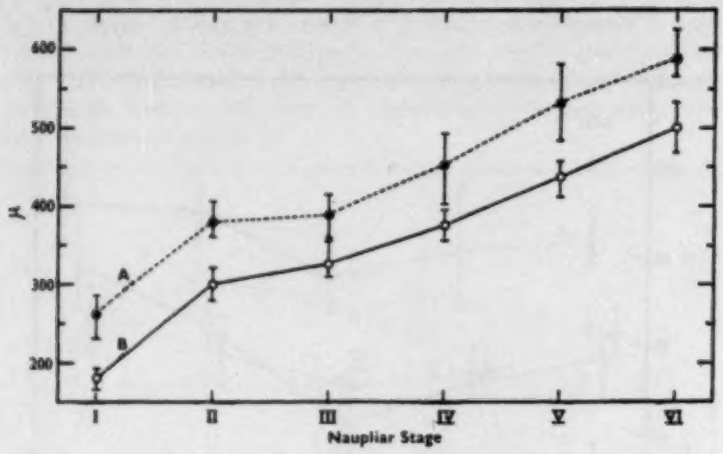

Fig. 10—Distance between tips of frontal horns in A, *Pyrgoma anglicum* ; B, *Balanus perforatus*. Means and extremes of ten individuals at each stage of both species.

(ii) Frontal horns hardly if at all forward of anterior edge ; furcal rami under 60μ.

(*a*) Small spines present on postero-lateral margin of body ; ratio length to breadth over 2·2 :—*B. perforatus*.

(*b*) A pair of protuberances sometimes visible, but no postero-lateral spines ; ratio length to breadth under 2 :—*P. anglicum*.

Stage IV.

(i) About five small spines on postero-lateral margin of carapace ; furcal rami over 65μ :-*A. spongites*.

(ii) No such spines ; furcal rami under 60μ.

(*a*) Ratio length to breadth under 1·9 ; distance between tips of frontal horns more than 400μ :—*P. anglicum*.

(*b*) Ratio length to breadth over 2 ; distance between tips of frontal horns under 400μ :—*B. perforatus*.

Stage V.

(i) About five small spines bordering carapace ; furcal rami over 80μ long :—*A. spongites*.

(ii) No such spines ; furcal rami under 80μ.

(*a*) Distance between tips of frontal horns over 475μ ; caudal spine slightly longer than abdominal process :—*P. anglicum*.

(*b*) Distance between tips of frontal horns under 475μ ; caudal spine considerably longer than abdominal process :—*B. perforatus*.

Stage VI.

(i) About fourteen small spines bordering carapace ; carapace spines over 110μ long :—*A. spongites*.

(ii) No such spines ; carapace spines under 100μ.
  (*a*) Caudal spine no longer than abdominal process ; ratio of length to breadth less than 1·7 *P. anglicum*.
  (*b*) Caudal spine considerably longer than abdominal process ; ratio of length to breadth over 1·7 *B. perforatus*.

THE CYPRIDS

These are very similar to those of other species. The numbers of setae and spines on the six pairs of thoracic limbs are the same as are found in other balanids, and so are the relative lengths of the setae. The pattern of setulation is the same and even the complex antennular suckers show more variation between individuals of different ages than occurs between young cyprids of different species. It has been observed with the present and other species reared in the laboratory that setae of the antennule become abraded or lost during the life of the cyprid, perhaps as a result of the use of these sensory structures in testing the substratum before settling. The similarity of the antennular suckers of *Acasta* and *Pyrgoma* cyprids to those of other balanid species is perhaps surprising in view of the atypical substrata on which they settle.

Fig. 11—Cyprids of A, *Acasta spongites* ; B, *Pyrgoma anglicum*.

The reared cyprids varied considerably in size (Table 1), like those obtained by Barnes (1953) from townettings. Figure 11 shows their appearance in outline. *Acasta* cyprids are shorter and relatively somewhat broader than those of *Pyrgoma* or *Balanus perforatus*. The sizes and proportions of these two last species, however, overlap considerably and no certain method of distinguishing between them has been devised, though *B. perforatus* cyprids are generally more slender.

ACKNOWLEDGMENTS

I would like to express my thanks to Dr Mary Parke of the Plymouth Laboratory for supplying me with phytoplankton cultures as food for the larvae, and to Professor Knight-Jones for introducing me to these animals underwater and for advice in the preparation of this account.

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RESPONSES TO DISTURBANCE OF THE PERIWINKLE *LITTORINA PUNCTATA* (GMELIN) ON A SHORE IN GHANA

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[Accepted 14th February 1961]

(With 3 figures in the text)

Specimens of *Littorina punctata* (Gmelin), the common periwinkle of Ghana, were subjected to a series of simple tests to see how they behaved when disturbed. The results suggested the initial tracks of freshly displaced winkles are compounded of two components, one directed up or down the shore, the other directed up or down a slope. In winkles collected from a vertical surface the upshore component is dominant on slopes below 90° ; above 90° (reverse slope) it is the up slope component. In those collected from the flat floor of a rock pool the down slope component is dominant on slopes of 30° or more ; on a flat surface the upshore component is revealed. The upshore component is related to a visual response, the slope component is not.

Results on a dark night with no moon do not differ from those in full sunlight. Visual responses depend on the use of terrestrial rather than astronomical beacons for at least part of the time. If winkles ever use the sun as a directional guide they allow for ephemeral changes in azimuth and also for change of transit bearing from north to south. A heavy swell can quickly alter the level at which the winkles are found and can temporarily affect some of their responses to disturbance.

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INTRODUCTION

The methods by which a winkle population maintains its position on the shore are not yet fully understood, although promising progress has been made over the years towards the solution of this problem. A comprehensive compilation of the literature has been given by Newell (1958 a, b) and will not be repeated here. The present work, directed to an elucidation of the gravitational and visual responses of freshly disturbed winkles, has been prompted by Newell's capital discovery that these responses differ in winkles taken from flat and vertical surfaces.

Two main groups of experiments were carried out, one in which simple tests were made to discover whether winkles exposed on the shore could be stimulated into movement by conditions resembling the returning tide, the

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other to record their movements when newly placed on a sloping surface as may happen to a winkle which falls or is washed from its hold.

The commonest winkle on the shore of Ghana is *Littorina punctata* (Gmelin). It is the dominant animal of the upper shore (Gauld & Buchanan, 1959) the population being densest between M.T.L. and H.W.N., but extending above H.W.S. Using this species observations were made at Christiansborg (5° 33′ N, 0° 11′ W), about two miles east of Accra. Here the walls of Christiansborg castle rise from inter-tidal rocks raggedly exposed between banks of shifting sand (Fig. 1). Both walls and rocks support a considerable population of winkles. On the rocks the winkles occupy all gradients from

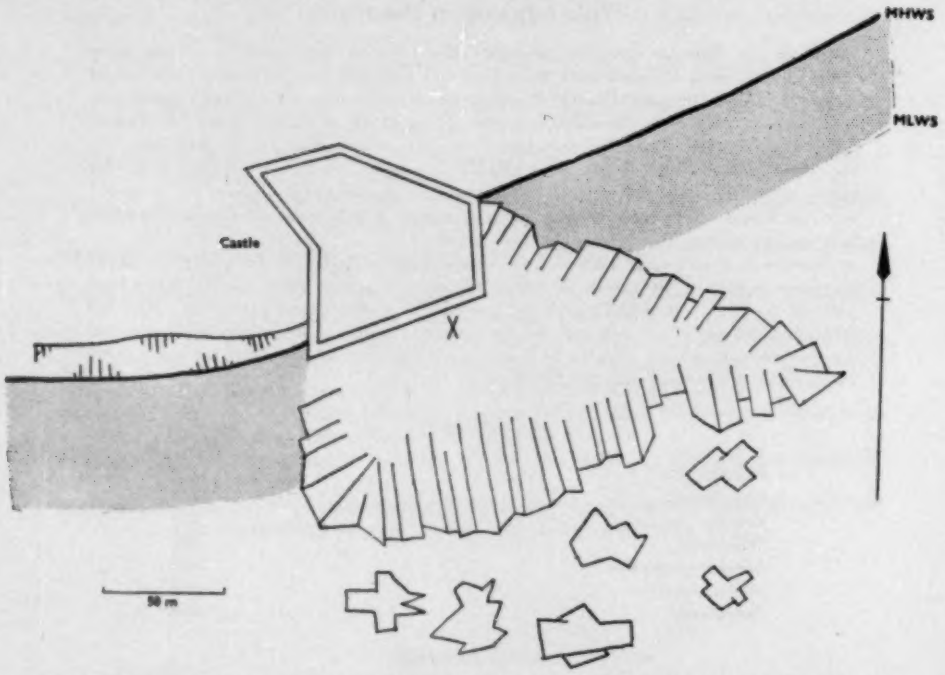

Fig. 1—The shore at Christiansborg, redrawn from Admiralty chart 3094, with acknowledgments to the Hydrographer of the Navy. X = the site of the investigation.

overhanging to flat and are common in the numerous rock pools. Many seek crevices where they aggregate. In this low latitude there is a great risk of desiccation and as the tide falls exposed winkles withdraw the foot and close the operculum, remaining attached only by a rim of mucus round the fore edge of the shell. On surfaces sloping less than about 30° they are randomly orientated, but above 30° they attach head uppermost. In rock pools many winkles remain active after the tide has fallen, persisting in slow crawling in all directions until it returns. Some, and these the larger ones, crawl out and settle just above the water level. For example on one occasion at a time of low water, 264 winkles, the whole population of a rock pool at the level of

H.W.N., had a mean shell length of 2·39 mm. The 110 winkles just above the rim of this pool had a mean shell length of 3·41 mm. By comparison, 161 winkles from the castle wall had a mean shell length of 6·10 mm., in other words, the smallest winkles on the upper shore favoured continual submergence, perhaps because their surface area/volume ratio exposed them to greater risk of desiccation.

EXPERIMENTS

First Group

In the first group of experiments, simulating the returning tide, the following observations were made. Winkles removed from a dry sloping surface and placed on another dry surface would reattach only after about 2 minutes. Winkles removed from a wave-washed surface reattached in 8 seconds or less. Winkles from a dry sloping surface reattached in about 8 seconds if first wetted. Addition of water to a small, dried out rock pool caused the winkles in it to move upwards. Addition of fresh sea water to a pool which had not dried out, sufficient to renew all the water in the pool, terminated all winkles movement for some minutes.

Winkles taken from the vertical wall of the castle and placed in a pool did not attempt to climb out but just attached by the foot. Winkles taken from the castle wall, when wetted, could be induced to climb dry rocks. Yet water repeatedly thrown over winkles resting undisturbed on the castle wall did not stir them.

The results of these experiments are somewhat inconclusive. There is some evidence that the returning tide subdues winkle movement (although this is contradicted by one observation) ; that winkles would rapidly reattach if knocked off by the first wave of the new tide ; and that if knocked off before the tide reached them they would slowly reattach and then crawl upwards.

Second Group

The second group of experiments caused the modification of some of these conclusions, particularly in the way that a winkle, disturbed from its hold and falling on to a slope would crawl. On an oblong board made of "Tufnol", about 40 cm. by 35 cm., nine circles each of 5 cm. radius were inscribed. Diameters were drawn at 20° intervals through each circle. The board was wetted in the sea and then laid on the shore. A winkle was placed in the centre of each circle. After the winkles had attached to the board by the foot the board was sloped to a predetermined angle; then as the winkles moved. the point at which they crossed the circumference of the circle was noted, Experience showed that by the time the winkles had reached the 5 cm. ring they had adopted a course which with few exceptions they followed to the edge of the board. A board inscribed with circles of 10 cm. radius was abandoned after trial as being time consuming without giving better results.

The shoreline at the foot of Christiansborg castle runs approximately east and west, with the shoreward side to the north. References to direction on a horizontal board are given in 360° notation, 000° being true north. On sloping

boards 000° refers to the higher side of the board, regardless of its direction. The terms " up slope " meaning up the slope of the board without reference to its orientation, and " upshore " (northwards) will be freely used, as will their opposites, down slope and downshore. They describe, in fact, the two major components of the winkles' tracks. A distinction is also to be made between winkles taken from the vertical surface of the castle walls (" wall winkles ") and those taken from the flat floors of rock pools (" pool winkles "). Only winkles from these situations were employed in the tests. No winkle was used more than once in any test.

RESULTS

From flat surfaces

Table 1 shows results obtained with the board laid flat on the shore about 2 métres from the castle wall. The winkle tracks fall clearly into two groups, those directed upshore and those directed downshore. Groups 1.1 and 1.2 are wall winkles. Here both the sea horizon and the morning sun were visible. The winkles moved upshore with little scatter, all tracks lying within 45° of the mean. The wall winkles in group 1·3 also moved upshore, this time in the light of an afternoon sun. Clearly, if the sun is used as a directional guide by the winkles they must be able to allow for a change in its azimuth. Further, comparing group 1.1 with group 1.4, collected about seven months later, it is seen that winkles can distinguish upshore from downshore whether the sun transits to the north or to the south of them. Wall winkles in group 1.5 were shaded from the sun but could see their distant surroundings below about

TABLE 1—Tracks of winkles freshly placed on a flat surface
Groups 1.1–1.9 are wall winkles, group 1.10 pool winkles

Group	Date 1959–60	Mean Track (°)	No. of winkles	No. within ±45° of mean	Sun's azimuth	Sun's mean altitude (°)
1.1	19th Nov.	345	10	10	SSE	62
1.2	4th Dec.	356	10	10	SSE	58
1.3	7th Dec.	354	20	19	WSW	33
1.4	28th June	341	20	20	N	72
1.5	7th Dec.	323	20	16	WSW	27
1.6	7th Dec.	010	20	19	WSW	20
1.7	19th Nov.	171	10	10	SSE	64
1.8	16th Dec.	212	10	10	SSE	58
1.9	16th Dec.	012	12	9	S	61
1.10	24th Feb.	347	20	18	ESE	42

15° elevation. Those in group 1·6 could see the sun, but by sinking the test board a little way into the sand they were prevented from seeing the sea horizon. In each case the winkles moved upshore. Wall winkles in groups 1.7 and 1.8 were shaded from direct sunlight but were shown the sun reflected from the opposite direction in a large mirror. In the first case they were able to see the sea horizon, in the second case not. All the winkles moved downshore, the reverse of previous directions, a strong indication that the upshore

component of the tracks derives from a visual response to the surroundings, in this instance the reflected sun and sky. Wall winkles tested on a board sunk into the sand and shaded from the sun (group 1.9) moved upshore. Here, where neither sun nor sea horizon was visible the winkles could see a part of the sky to seaward and the wall of the castle shoreward ; visible orientation was perhaps still possible for them.

Winkles from the flat bottom of a rock pool showed the same response to being laid on the test board as wall winkles ; with the sun and horizon both visible the pool winkles in group 1.10 crawled upshore.

From sloping surfaces facing east and west

So far the results have shown no distinction between the responses of wall winkles and those of pool winkles, but a distinction begins to appear in the results of Table 2, when the board was sloped. Here the face of the board was set to the east or west, and the track angles (0°–360°) are now given with respect to the high side of the board so that a winkle crawling up slope has a track angle of 0° regardless of compass direction.

Groups 2.1–2.3 are of wall winkles on an east facing board. On slopes of 50° and 70° they moved horizontally upshore, at 90° they moved both upshore and up slope. On a west facing board sloped at 30° (group 2.4) wall winkles moved upshore but hardly at all up slope. The pool winkles in groups 2.5 and 2.6 have tracks compounded of upshore and downslope components. The sun was concealed behind cloud in groups 2.2 and 2.5, otherwise sun and sea horizon were visible throughout these tests.

TABLE 2—Tracks of winkles freshly placed on sloping surfaces facing east or west
Groups 2.1–2.4 are wall winkles, groups 2.5 and 2.6 pool winkles

Group	Date 1959	Slope (°)	Face of Board	Mean Track (°)	No. of winkles	No. within ±45° of mean	Sun's azimuth	Sun's mean altitude (°)
2.1	3rd Dec.	50	East	090	20	19	S	62
2.2	3rd Dec.	70	East	087	20	20	SE	50
2.3	20th Nov.	90	East	032	10	10	SSE	62
2.4	4th Dec.	30	West	273	10	10	SSE	61
2.5	3rd Dec.	70	East	120	20	17	SSE	56
2.6	20th Nov.	90	East	160	10	9	S	65

Table 2 suggests that when a board slopes *across* the upshore direction then provided the slope is steep enough wall winkles will move both upshore and up slope, while pool winkles will move upshore but down slope.

From sloping surfaces facing south (or downshore)

By varying the facing and slope of the board it is possible to cause the components upshore and up slope to support or oppose each other. On a south facing board, on slopes from 0–90° they are combined ; past 90° they are opposed ; on a north facing board the reverse is true.

Table 3 refers to a south facing board. Groups 3.1–3.7 are of wall winkles, 3.8–3.14 of pool winkles. All the wall winkles crawled up slope and all the

pool winkles crawled down slope. The upshore component, where it opposed the slope component (below 90° for pool winkles, above 90° for wall winkles) did not reverse the track direction and indeed appears quite ineffective in the Table 3 results.

TABLE 3—Tracks of winkles freshly placed on sloping surfaces facing south
Groups 3.1–3.7 are wall winkles, 3.8–3.14 pool winkles

Group	Date 1960	Slope (°)	Mean Track (°)	No. of winkles	No. within ±45° of mean
3.1	16th Jan.	30	000	20	20
3.2	,,	50	353	20	17
3.3	,,	70	337	20	12
3.4	,,	90	352	20	14
3.5	17th Jan.	110	355	20	15
3.6	,,	130	003	20	13
3.7	,,	150	348	20	14
3.8	24th Feb.	30	156	20	13
3.9	,,	50	148	20	12
3.10	,,	70	195	20	17
3.11	,,	90	181	20	19
3.12	,,	110	184	20	19
3.13	,,	130	190	20	17
3.14	,,	150	187	20	19

TABLE 4—Tracks of winkles freshly placed on sloping surfaces facing north
Groups 4.1–4.11 are wall winkles, 4.12–4.18 pool winkles

Group	Date 1960	Slope (°)	Mean Track (°)	No. of winkles	No. within ±45° of mean
4.1	12th Feb.	30	158	20	16
4.2	16th June	30	169	20	17
4.3	12th Feb.	50	160	20	19
4.4	16th June	50	169	20	16
4.5	16th June	70	150	20	14
4.6	28th June	70	200	20	17
4.7	12th Feb.	90	306	20	9
4.8	16th June	90	071	20	6
4.9	28th June	110	330	20	15
4.10	28th June	130	342	20	17
4.11	28th June	150	343	20	18
4.12	12th Feb.	30	174	20	20
4.13	12th Feb.	50	170	20	18
4.14	12th Feb.	70	162	20	18
4.15	12th Feb.	90	166	20	10
4.16	28th June	110	180	20	18
4.17	28th June	130	183	20	17
4.18	28th June	150	184	20	16

From sloping surfaces facing north (or upshore)

In Table 4, however, its presence becomes evident. 4.1–4.11 are wall winkles, 4.12–4.18 pool winkles ; the board faced north. The first six examples

show wall winkles moving upshore but *down slope*. At 90° the slope response is hesitatingly reversed. Past 90° the two components are united and the winkles are able to move both upshore and upslope. Tests on slopes of 30°–90° were duplicated in view of the critical nature of the results. The pool winkles again crawled solidly down slope.

The results of Tables 3 and 4 and part of Table 1 are summarized diagrammatically in Figure 2. From it, it may be concluded that pool winkles always crawl down slope when the slope is 30° or more, but on a flat surface they move upshore. Below 90° wall winkles move upshore but above this angle they move up slope.

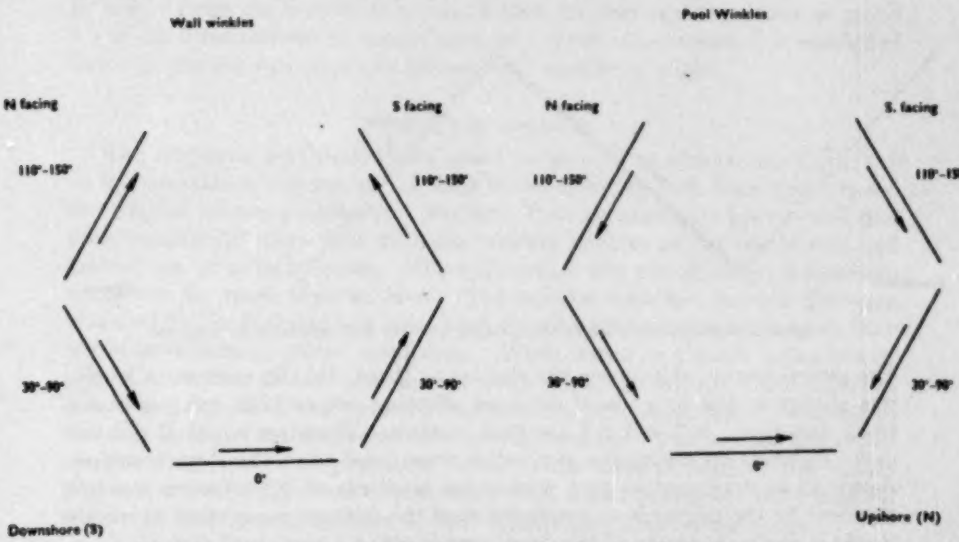

Fig. 2—Diagrammatic summary of the movements of pool and wall winkles on boards facing upshore and downshore.

The underside of flat surfaces

Numerous attempts were made to get the winkles to crawl on the underside of " Tufnol " boards and glass sheets held flat, but they usually fell off and there was no predominant track of those that did not. The track direction of the winkles on 180° slopes therefore remains unknown.

Responses to light and gravity

It is possible to demonstrate that the upshore component of the winkles' tracks is dependent on a visual response and that the slope component is not. (It is almost certainly gravitational.) Figure 3 shows two boards sunk in the sand with a mirror separating them. Both boards faced north and sloped at 50°. Winkles on board B were able to see the sky (but not the sun) reflected in the mirror, for the boards lay in the shadow of the castle wall. Results of four groups of tests are given in Table 5. Groups 5.1 and 5.2 are wall winkles. The first group, on board A, moved down slope and upshore as expected.

TABLE 5—Tracks on boards arranged as in Fig. 1. Board A without mirror, board B with mirror

Groups 5.1 and 5.2 are wall winkles, 5.3 and 5.4 are pool winkles
30th June, 1960

Group	Board	Mean Track (°)	No. of winkles	No. within ±45° of mean
5.1	A	181	20	16
5.2	B	348	20	18
5.3	A	180	20	18
5.4	B	160	20	16

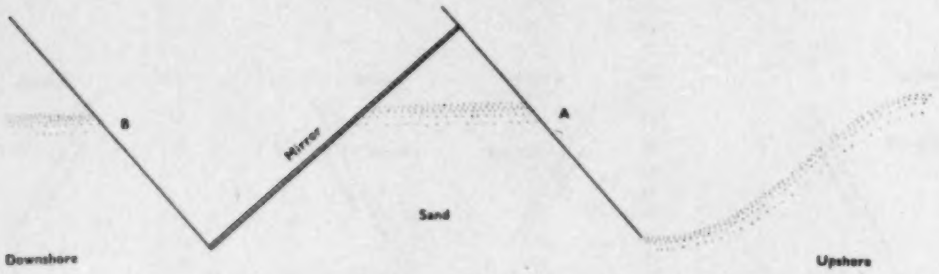

Fig. 3—Boards partly sunk in sand, facing upshore and separated by a mirror.

The second group, able to see the sky below them, did the reverse. Clearly, this change is due to a visual response affecting one or both components of track direction. 5.3 and 5.4 are pool winkles. Those on board B did not move upwards on seeing the sky below them, and since the slope response, shown to be dominant in pool winkles on gradients of 30° or more, was not reversed by the mirror it is concluded that the upshore component of winkle tracks is due to a visual and the slope component to a non-visual factor.

Responses in the dark

Experiments were conducted to discover the effect of reduced light on the winkles' responses. On a night of no moon with low cloud (29th January, 1960) twenty wall winkles moved upshore on a horizontal board ; twenty more did the same on a board sunk flat into the sand and hence with the sea horizon obscured. Similar results were obtained with equal numbers of pool winkles. On a vertical board facing east ten wall winkles moved up slope and upshore on a mean course of about 020° (exact tracking was difficult in the dark). On a vertical board facing west ten wall winkles did the same, on a mean course of about 340°. Nine out of ten pool winkles on a vertical board facing west moved down slope and upshore (about 200°) but on an east facing vertical board ten pool winkles moved up slope (about 050°). This last result does not accord with either earlier or later observations of pool winkles' behaviour and is recorded here without explanation. Otherwise the results are what would be expected by day. On the night of 9th June, 1960, with a full moon hidden behind cloud, tests were made with north and south facing boards sloping at

TABLE 6—Tracks on boards facing north or south and sloping at 50°
Groups 6.1 and 6.3 are wall winkles, 6.2 and 6.4 are pool winkles
The night of 9th June, 1960

Group	Face of Board	No. of winkles	No. of tracks up slope	No. of tracks down slope
6.1	North	20	5	15
6.2	North	20	3	17
6.3	South	20	16	4
6.4	South	20	2	18

50°. The results appear in Table 6 ; again they are what would be expected by day. Since the horizontal tracks of 29th January and the tracks of group 6.1 of 9th June contain dominant upshore (visual) components it is concluded that the winkles can orientate themselves visually by night.

Stability of responses

The responses described above could be elicited at almost any time, but on one occasion it was not so. A visit to the shore on 18th June, 1960, found the winkles behaving normally. On 20th June an unusually heavy swell had been running for more than 24 hours and the winkles on the castle wall had moved 1 m. or more upwards. The wall surface was wet although it had been uncovered for more than an hour. The winkles were not moving but were attached by the foot and not lying head up with the operculum closed as they would have been in quieter conditions. When tested on a north facing board, first at 110°, then at 150° they moved down slope, the reverse of the expected direction. By 21st June the swell had subsided, the winkles had returned to their old level and had recovered their usual responses. The unusual behaviour of 20th June was most probably linked to the shift in level of the winkle population ; crawling down slopes which they would previously have crawled up would tend to restore them to their former positions on the shore.

DISCUSSION

The initial responses to disturbance described in this account are not in themselves sufficient to maintain a winkle population in a particular zone. Clearly, at different times the same winkle must move up and down the shore and up and down slopes. Again, winkles which stop moving at the return of the tide must later begin to crawl and feed. Perhaps the main significance of the present work lies in two points. Firstly it has shown in *Littorina punctata* the " wall winkle " and " pool winkle " responses first discovered by Newell in *L. littorea*. Secondly, it has distinguished in these responses a non-visual (gravitational) component and a visual component.

The non-visual component presents little difficulty of explanation ; but finding the visual component leaves unanswered the question of what the winkles are seeing and responding to. There is good evidence that *L. littorea* makes use of the sun as a compass without, however, allowing for changes in its azimuth (Newell, 1958, b). Burdon-Jones & Charles (1958) have demonstrated responses to plane polarized light in *L. littoralis*. But neither of these

visual responses can be of value at night ; indeed Baylor (1959) has pointed out that " the invocation of polarized light as a navigational aid is probably unnecessary because usually when the sky is strongly polarized the sun is visible ".

Williamson (1951) thought that *Talitrus saltator* was directed upshore after its nocturnal forays by the aid of form vision. He noted that " there are very few summer nights when a silhouette of the sand dunes against the sky is not visible to man from the beach ". This is true of the coastline at Christiansborg and offers a likely explanation of what is seen there by the winkles at night. This much can be said with certainty, that their visual responses depend on the use of terrestrial rather than astronomical objects for at least part of the time.

ACKNOWLEDGMENTS

I am indebted to my colleagues, G. E. Newell for drawing my attention to this problem and for much subsequent advice, and J. B. Buchanan who read the manuscript.

Additional note

Since writing this account I have been able to inspect a series of sections which Newell has prepared of the eye of *Littorina littorea*. First examination suggests that this winkle is well able to form visual images and perhaps to focus the eye at various distances. Form vision, of course, presupposes the possession of such an eye.

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THE ANATOMY OF THE NERVOUS SYSTEM OF THE MESOTHORAX OF *LOCUSTA MIGRATORIA MIGRADORIOIDES* R. & F.

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(With 10 figures in the text)

A description is given of those nerves which arise from the mesothoracic ganglion of *Locusta migratoria migratorioides* R. & F. There are six pairs of nerves, of which the sixth and most posterior are referred to as recurrent nerves since they join the first nerve of the ganglion in the metathorax. The median nerve is also described. The posterior median nerve of the prothoracic ganglion is noted as its branches make a connection with the branches of the first mesothoracic nerve. Wherever possible an attempt has been made to relate the observations to those of some other authors.

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INTRODUCTION

The African migratory locust (*Locusta migratoria migratorioides* R. & F.) and the desert locust (*Schistocerca gregaria* Forsk.) are now extensively used in physiological studies. As there has not been a sufficiently detailed study of the nervous system of these animals it has become desirable to make more complete investigations and drawings of the nervous system.

A general anatomy of the migratory locust has been published (Albrecht, 1953), but the section dealing with the nervous system is inadequate for modern physiological investigations. D. W. Ewer (1954 a) has described in some detail

the innervation of the mesothorax of the tree locust, *Acanthacris ruficornis* (Fab.), but he did not include a description of those nerves supplying the femur.

It was decided to start with the anatomy of the nervous system of the mesothorax of the migratory locust. This species was made readily available by the Anti-Locust Research Centre in London. The mesothorax is the least specialized segment of the thorax ; it contains wing muscles and the mesothoracic ganglion is discrete and unlike that of the metathorax which is combined with the ganglion of the first abdominal segment.

METHODS

Adult locusts were dissected from various aspects depending on whether the nerves being studied were ventral or dorsal in their courses. After decapitation some were dissected from the ventral surface ; others were opened along the mid-dorsal line and the gut removed to obtain views of the dorsal surface of the ganglion and routes of the nerves in the pleural regions of the thorax. About twenty minutes before dissection, the specimen which was to be examined was injected with a solution composed of 50 per cent 0·1 per cent methylene blue and 50 per cent locust saline (Hoyle, 1953). This locust saline was used as immersion fluid at the beginning of the dissection. Sometimes the fixative ammonium molybdate (10 per cent solution) was used, but unfortunately, this made the muscles and other tissues very brittle and thus difficult to dissect. At other times the specimens were pinned out and fixed in Bouin and afterwards preserved in 70 per cent alcohol. Parts of the nervous system could then be dissected out and stained, using either haematoxylin (Heidenhain) or acid fuchsin and aniline blue/orange G (Mallory) ; the preparations thus obtained were used to confirm the results produced by injecting the methylene blue intra-vitally. These methods were necessary when investigating the structure of the chordotonal organ of the mesothorax so that distinction could be made between the skeletal and the nervous parts of the structure.

NOMENCLATURE

Many studies have been made of the insect muscular system (Weber, 1928 ; Snodgrass, 1929 ; Maki, 1938 and Carbonell, 1947). However, although several species have been studied, and although the systems of nomenclature are various, there is a recognizable basic plan in the pattern of insect musculature. In this study the terminology of the muscles follows that of Snodgrass (1929), however, there are additional muscles, not mentioned by Snodgrass, which are found in the newly-moulted adult. Where they have been observed in this investigation reference has been made to the numbers given to them by Maki (1938) and also to the names given by Ewer (1954 c) to the equivalent muscles in *Acanthacris ruficornis* (Fab.)

Origins and insertions of muscles have not been included in the general report as these details are given in the Table 1 (see pp. 420–425).

In this study the paired nerves of the mesothoracic ganglion have been numbered, antero-posteriorly 1–5, while the sixth pair has been called

recurrent, since there is a connection with the first pair of the metathoracic ganglion. The branches of the median dorsal nerve which innervate the spiracular muscle have been named spiracular nerves. The first branches of the nerves 1–5 are designated by the addition of capital letters (A, B, C, etc.); the secondary branches are designated by small Arabic numerals (e.g. $3B_2$); further ramifications are indicated by the addition of a small letter of the alphabet (e.g. $3B_2c$). The first branches of the recurrent nerves have been distinguished by small letters, e.g. M.Rn.a, the prefix "M" indicating that the nerve emanates from the mesothoracic ganglion; the secondary branches by small numerals. The latter method, being slightly different from that used for nerves 1–5, has been used to differentiate the recurrent nerve.

OBSERVATIONS

For convenience, nerves 1, 2, 3, 4 and 5, the recurrent nerve, and branches of the median nerves of the prothoracic and mesothoracic ganglia, are each described singly; in fact they are paired, one of each pair supplying the apposite half of the segment.

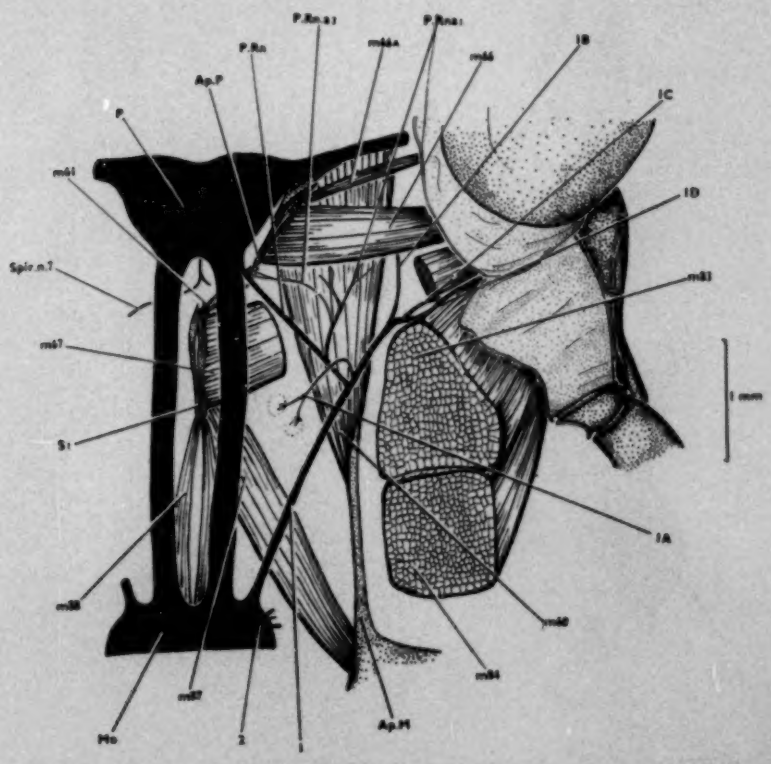

Fig. 1—Ventral view of Nerve 1 of the left side of the mesothoracic ganglion. (Key to lettering, p. 431)

Nerve 1

Nerve 1 emerges from the anterior border of the mesothoracic ganglion at an angle of about 45° in the direction of the prothoracic coxa and, near the tip of the anterior projection of the mesosternal apophysis, is joined by the

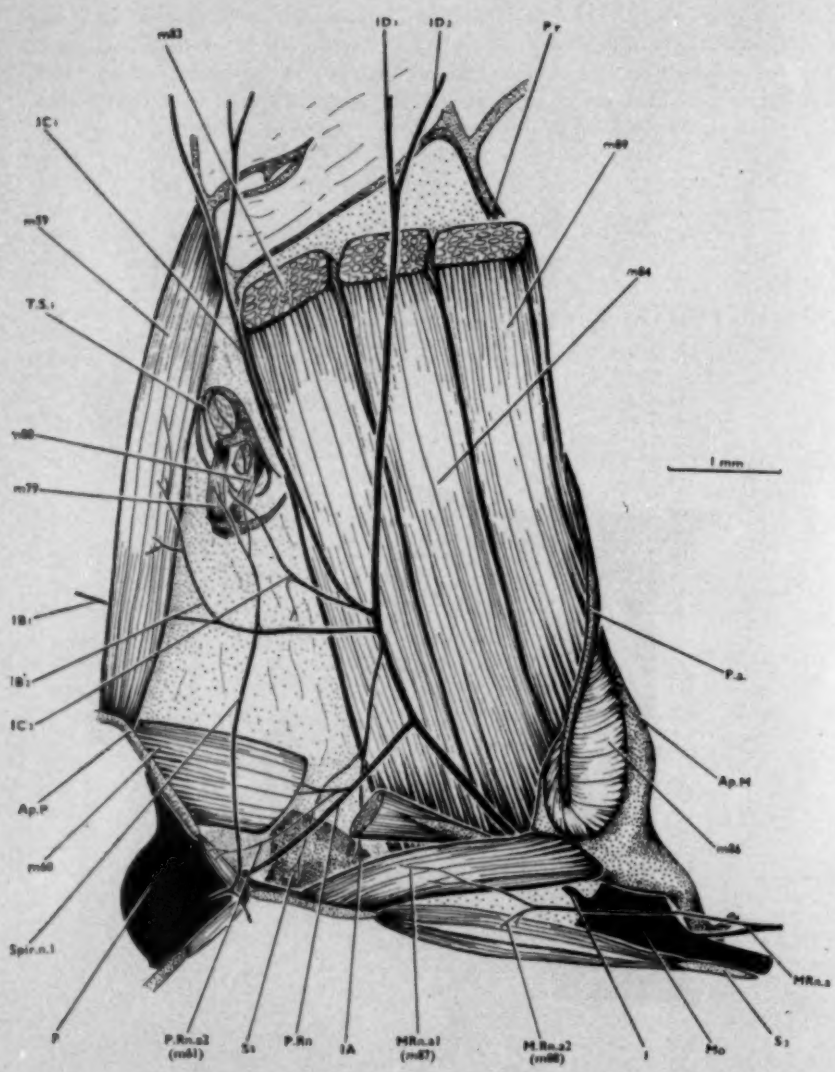

Fig. 2—Inner view of part of the right side of the thorax, showing Nerve 1 of the right side of the mesothoracic ganglion. (Key to lettering, p. 431)

sixth or recurrent nerve from the prothoracic ganglion (Fig. 1, P.Rn.). From P.Rn., at a point two-thirds of the distance from the prothoracic ganglion to nerve 1 of the mesothorax, there is a branch, P.Rn.a, which runs anteriorly, one limb of which, P.Rn.a_1, innervates muscle 60 and the other, P.Rn.a_2, innervates the small sternospinal muscle 61. 1A separates from nerve 1 just beyond the junction of P.Rn with 1 ; it is a small sensory nerve which supplies the region of the spinasternum. There are then three main branches of 1. The first, 1B, runs anteriorly dorsal to m66 (Fig. 1) and has three branches ; there is $1B_1$ (Fig. 2) which runs into the prothorax and joins the branch 1B of the prothoracic first nerve, which innervates the protergal muscles of the cervical sclerites (52a & b and 53), and $1B_2$ which innervates muscle 59 (intersegmental). There is also a very small branch, $1B_3$ (Fig. 2, not labelled due to position in drawing), which joins the spiracular nerve from the prothoracic ganglion : this will be discussed at a further stage in this report. The second and third of the three main branches of 1 turn dorsally (Fig. 2, 1C and 1D). A small branch of 1C innervates the membrane between the prothoracic and mesothoracic pleurites and also the peritreme of the first thoracic spiracle ($1C_2$). The larger part, $1C_1$, has three main branches ; the most dorsal branch, $1C_1c$, goes to the region of the first phragma (Fig. 8), the second $1C_1b$, goes to the region of the wing base and the other, $1C_1a$, innervates the costal region of the wing. The first branch of 1D innervates the muscles 81 and 82 (Fig. 8, $1D_1a$) and also the region of the second phragma ($1D_1b$). The second, $1D_2$ innervates the region of cuticle round the fourth axillary sclerite.

Nerve 2

Nerve 2 is a thin sensory nerve from the ventral surface of the ganglion and has three main branches (Fig. 3, 2A, 2B, 2C), sometimes appearing as three separate roots. The branch 2A and some fibres of 2C ($2C_1$, $2C_3$ and $2C_4$—the first two are not labelled in Fig. 3) innervate the four groups of sense cells of the mesothoracic chordotonal organ. This organ is found in the mesosternal region of the thorax, one in each lateral half of the segment, and is in close association with branches of the second nerve. Strands of semi-sclerotised endoskeleton connect each of the four areas containing sense cells to parts of the sternal area, spreading out in a fan-like shape. The most anterior strand (SO) is inserted on the membrane between the prosternum and mesosternum lateral to the first spina ; the second is inserted on the basisternum (Ba) ; the third, on the membrane adjacent to the coxal rim (Cx) ; and the fourth, posteriorly-directed strand is attached to the sternellum (St). The sense cells of the first strand (SO) are connected to the other three sensory areas by similar strands ; also, the second is connected to the third (Fig. 3). Other branches of nerve 2 innervate the basisternum (2B, $2C_2$) and the posterior projection of the mesosternal apophysis ($2C_5$). This part of the anatomy was studied from both dorsal and ventral aspects, but the drawing is of the ventral view and thus the parts encircled by dotted lines are those which terminate on that part of the sternum which has been cut away in dissection.

Nerve 3

Nerve 3 is a relatively large nerve, emerging dorsal to nerve 2, roughly parallel with the long axis. There are three main branches. One runs anteriorly and dorsally (Fig. 4, 3A) along the inner face of the tergosternal muscle, number 84 (Fig. 5) giving off four branches at the same point ; the most anterior, $3A_1$, goes to the muscles 83 and 84 ; the next, $3A_2$, goes to the muscles 97 and 98 ; the third branch innervates the three muscles lying in the episternum, 94, 95 and 96 ; and 3_4 goes to muscle 89 (Figs. 5 and 8). The second branch of the third nerve travels towards the meso-coxa and, at the edge of the sternum, divides into three branches which are mainly sensory. The first, $3B_1$, innervates the region of the sternum and episternum round the anterior rim of the coxa and the third, $3B_2$, innervates the region of the coxal

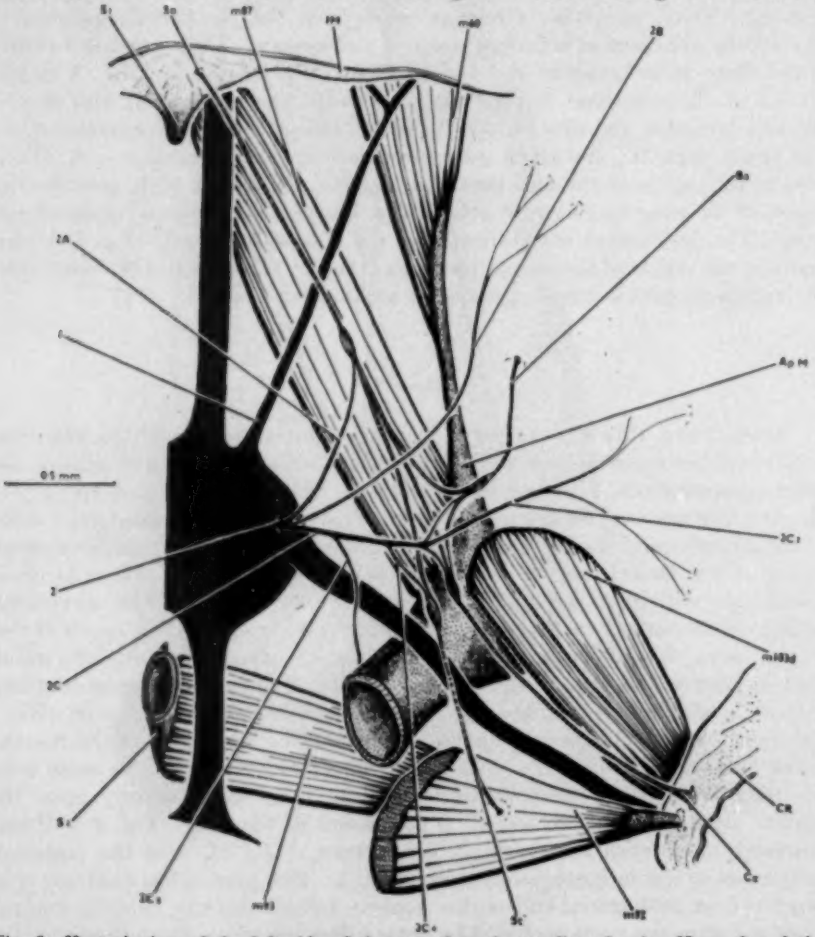

Fig. 3—Ventral view of the left side of the mesothoracic ganglion showing Nerve 2 and the associated chordotonal organ. (Key to lettering, p. 431)

membrane round the trochantin (Fig. 4). The second branch, $3B_2$, which enters the coxa, divides into three parts. The first part, $3B_2a$, innervates the anterior rim of the coxa and the second, $3B_2b$, innervates the dorsal coxal sense organ (hair plate). Finally, the third branch, $3B_2c$, enters the trochanter where it joins a branch of the fifth nerve ; this will be discussed when describing nerve 5. The third and posterior branch of the second nerve has three branches ; $3C_1$ travels posteriorly ventral to the projection of the mesosternal apophysis and innervates muscle 92 (Fig. 5) ; $3C_2$ supplies one of the muscles of the group which depresses the trochanter (Fig. 4 and Fig. 5, 103d) ; $3C_3$ runs ventrally over 103d, turning dorsally at a point between m103d and m89, continuing until it enters the units of the depressor of the trochanter which have their origins on the scutum (103 b+c, Figs. 5, 7 and 8). In the newly-moulted adult there is a muscle called by Ewer (1954 a and 1955) the coxa-spinal rudiment, which has been given a provisional number 92A, in Table 1, but is not included in any of the drawings. It is supplied by a very thin branch of $3C_3$ which has been called $3C_3a$ in Table 1. This muscle and the nerve ($3C_3a$) disappear a few days after the final moult.

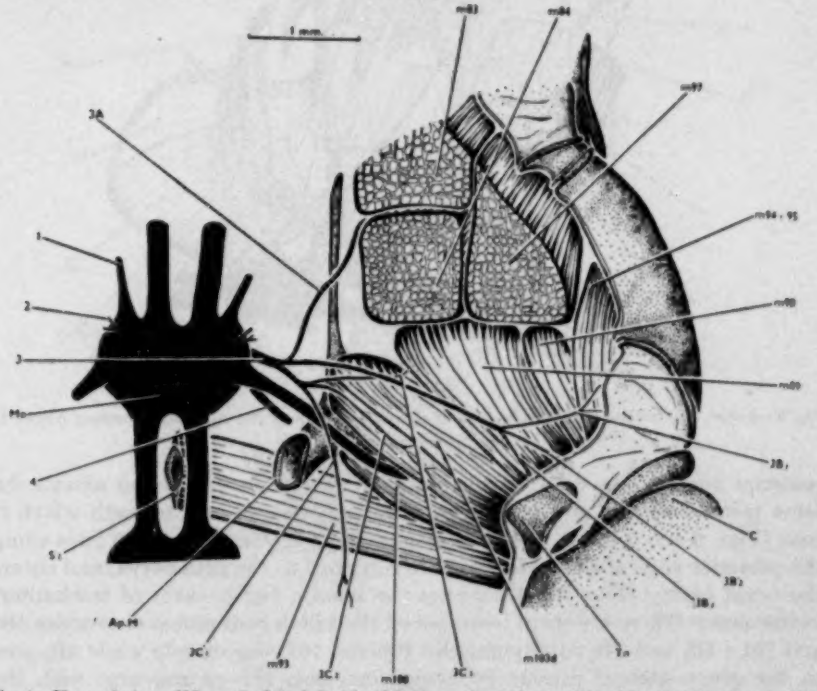

Fig. 4—Ventral view of Nerve 3 of the left side of the mesothoracic ganglion. (Key to lettering, p.431)

Nerve 4

Nerve 4 emerges slightly anterior and dorsal to nerve 5 at an angle of about 30° to the long axis and, crossing the root of nerve 5, runs towards the

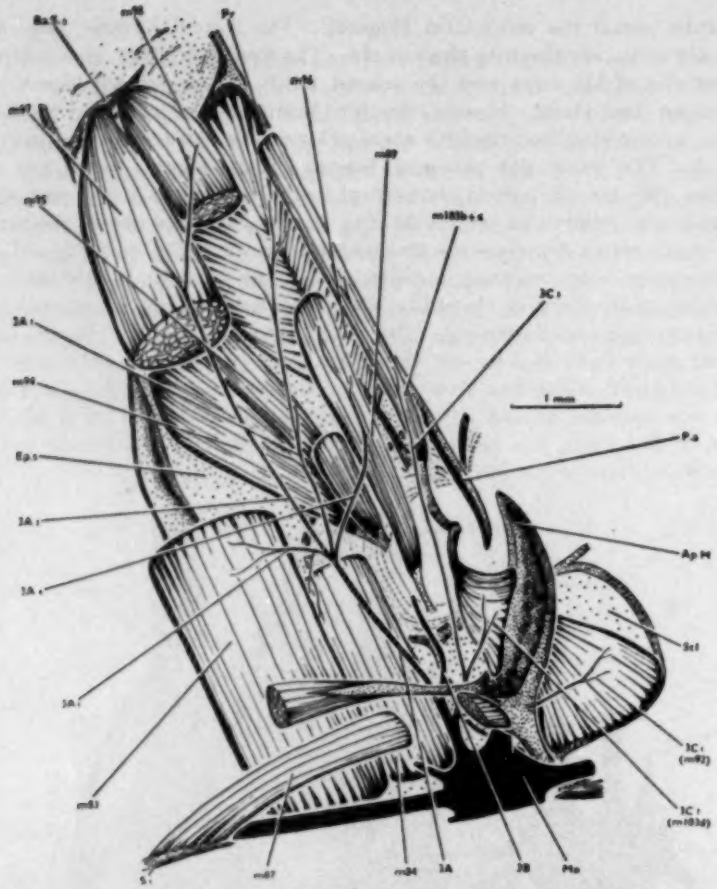

Fig. 5—Inner view of the episternal region of the mesothorax of the right side showing Nerve 3. (Key to lettering, p. 431)

posterior angle of the coxa. Four branches arise from it, often at almost the same point, near the posterior edge of the mesosternal arm beneath which it runs (Figs. 6 and 8 ; N.B. Fig. 7 is a ventral view). The branch 4A runs along the posterior edge of the mesosternal arm, dorsal to the fifth nerve, and enters the coxal cavity (Fig. 7) ; it supplies the muscle 102 (levator of trochanter) in the coxa. There are three branches of 4B, which runs dorsal to muscles 100 and 101 ; $4B_1$ and $4B_2$ supply muscles 100 and 101 respectively while $4B_3$ goes to the pleuro-sternal muscle 86, which connects the pleural arm with the dorsal surface of the lateral projection of the mesosternal apophysis. The anterior rotator of the coxa (m92) is innervated by the third branch, 4C, which runs on to the dorsal surface of the muscle. A large branch of the fourth nerve, 4D, runs posteriorly, ventral to muscle 93 and then curves dorsally into the region of the epimeron. The muscles in this region are m90, supplied by

$4D_1$; m91, supplied by $4D_2$; m99, supplied by $4D_3$; and m85 supplied by $4D_4$ (Fig. 6).

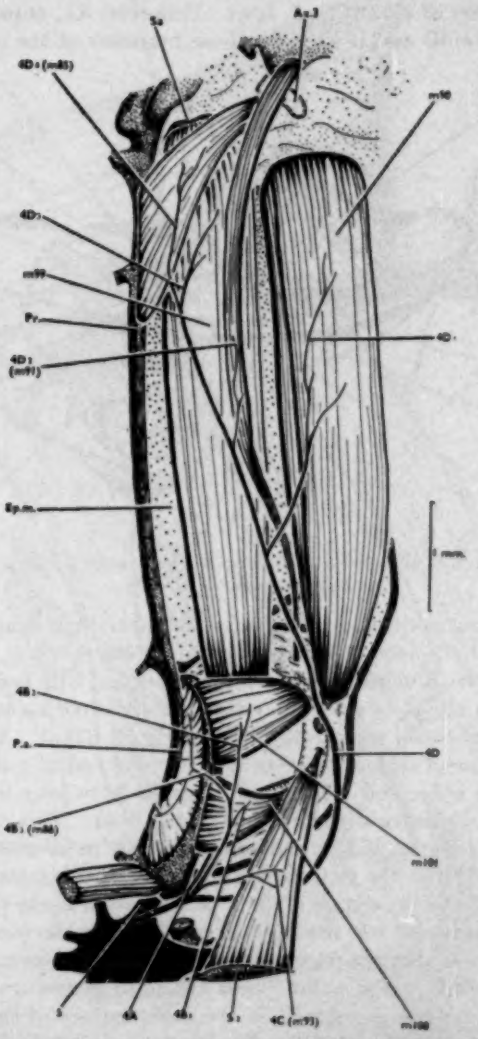

Fig. 6—Inner view of the epimeral region of the right side of the mesothoracic segment, showing Nerve 4. (Key to lettering, p. 431)

Nerve 5

Nerve 5, emerging ventrally and posterior to nerve 4, runs towards the coxal cavity (Fig. 7, ventral view) between the group of muscles 103 (b+c+d) and the group 100+101. The first branch, 5a is relatively small and diverges at the point where the main nerve passes underneath the posterior projection

of the mesosternal apophysis. The posterior ventral rim of the coxa (cut away in Fig. 7) is innervated by a small branch of 5A which runs ventrally round the insertion of m92 (Fig. 7, $5A_1$). However, $5A_2$ enters the coxa close to the main nerve 5B and it supplies those branches of the group of muscles

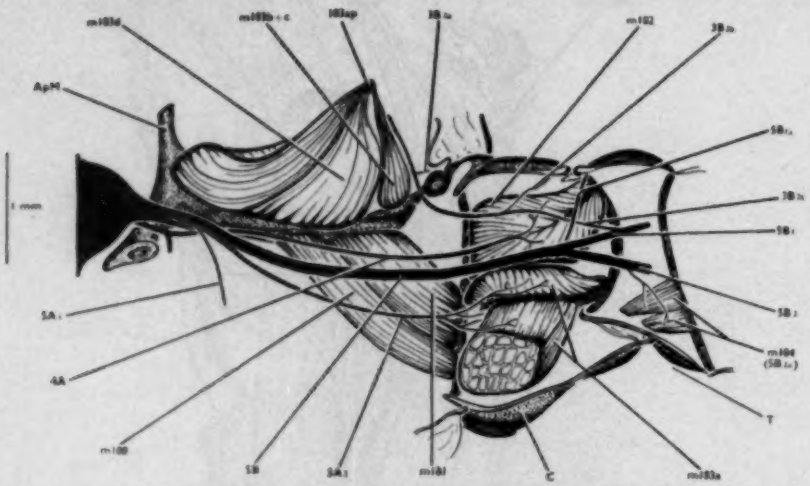

Fig. 7—Ventral view of Nerve 5 as it enters the coxa and trochanter of the left side.
(Key to lettering, p. 431)

termed the depressors of the trochanter, which have their origins in the ventro-posterior wall of the coxa. At approximately the level of the basal rim of the trochanter, 5B divides into two main branches, $5B_1$ being dorsal to $5B_2$. The first branch of $5B_1$ is sensory and supplies the cuticle of the base of the trochanter in the dorsal region (Fig. 7, and Fig. 9, $5B_1a$). At the base of the femur and the dorsal surface there is attached one end of a chordotonal organ (CO, Fig. 9) the other end of which is inserted by a long tendon on a small sclerite which articulates with the base of the tibia. This organ is described in *Melanoplus differentialis* by Slifer (1935) and in *Mecostethus grossus* by Debaisieux (1938), but the structure is identified as the anterior levator of the tibia (muscle 105) by Snodgrass (1929). It does seem likely that this structure was originally muscular but has become modified for the purpose of detecting changes of tension in tibia-femur relationships. In *Locusta migratoria* it is innervated by $5B_1b$ at the point where the most concentrated region of the sense cells of the organ is inserted on the inner surface of the skeleton of the base of the femur. Slifer (1935) calls this region of sense cells the proximal scoloparium, which is made up of units called scolophores. Associated with the proximal scoloparium and attached to it by several strands of connective tissue is the distal scoloparium (Slifer, 1935), a structure containing many large sense cells supplied by nerve $5B_1c$. This part of the chordotonal organ has several " stretch " fibres which connect it with muscles 107a and 107b, and with the dorsal part of the inner surface of the skeleton of the femur. The latter connection is shown, but not labelled in Fig. 9, and the former connec-

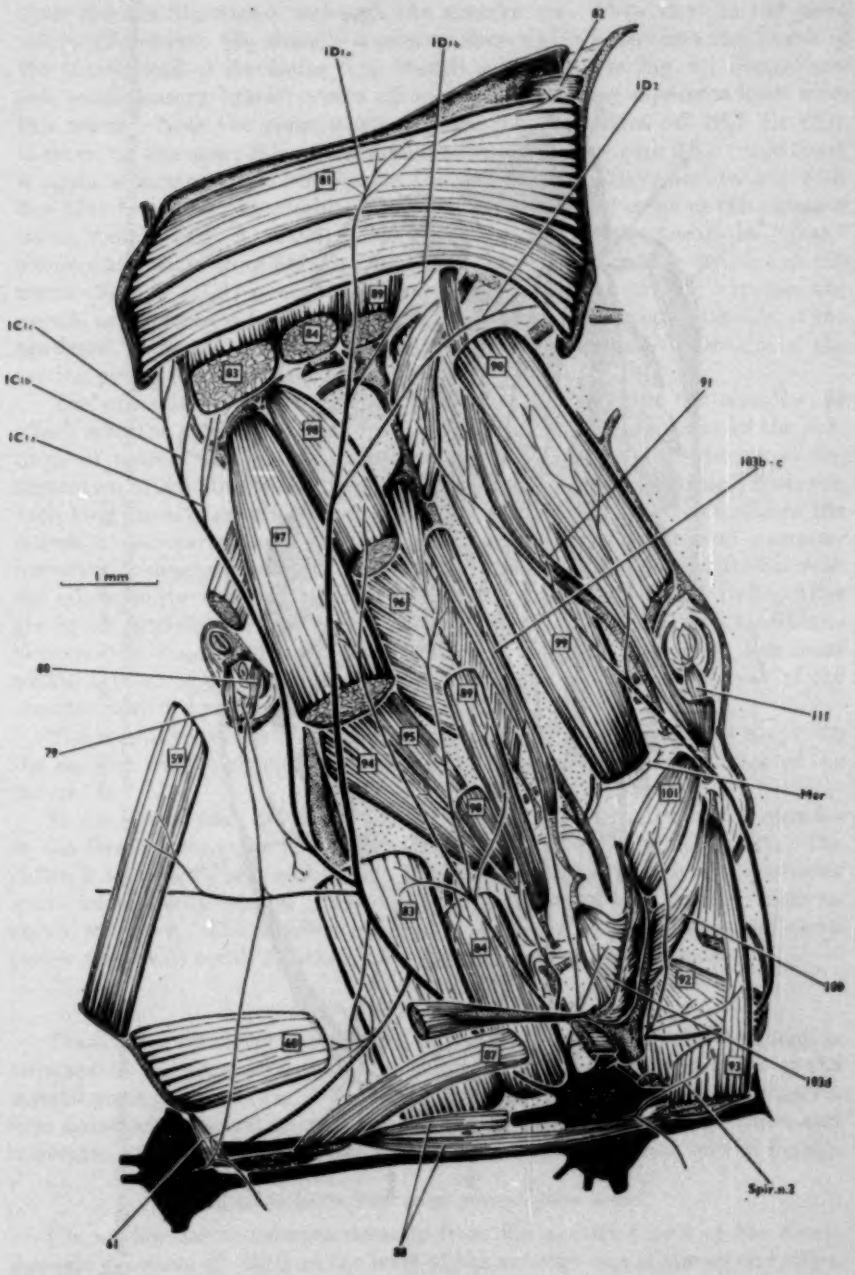

Fig. 8—General view of the right side of the mesothorax. (Key to lettering, p. 431)

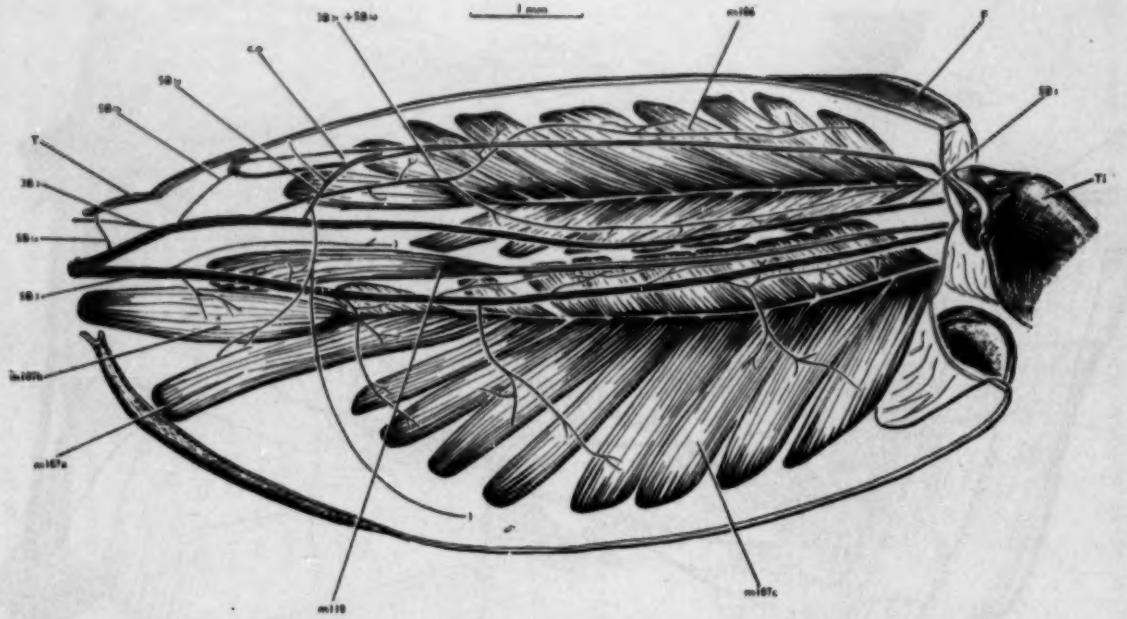

Fig. 9—The left femur which has been cut along the dorsal edge of the anterior wall, the latter having been deflected to show the branches of Nerve 5. (Key to lettering, p 431.)

tions are not illustrated, although the muscles are. Very close to the point where $5B_1c$ leaves $5B_1$ there is a sensory fibre which innervates the length of the frontal wall of the femur (one branch is cut short in Fig. 9). Sometimes this small sensory branch comes off with $5B_1c$ and then separates itself from this nerve. Near the point where, on one side, $5B_1$ gives off $5B_1b$ (to CO), there is, on the other side of the main nerve, a junction with $3B_2c$; this nerve is again separated from $5B_1$ (before the departure of $5B_1c$) and taking with it a fibre from $5B_1$, the combination ($3B_2c + 5B_1d$) then supplies the extensor tibiae muscle (Fig. 9, m106). The branches from $3B_2c$ contain the "fast" axons while those from 5B are "slow" (Hoyle, 1957). In Fig. 9 although the nerve ($3B_2c + 5B_1d$) is shown bifurcating so that one branch supplies the muscle units of one side and the other supplies those of the opposite side of the apodeme, in many specimens the single nerve runs along the length of the tendon giving off branches to either side.

The other large branch of the fifth nerve, $5B_2$, supplies the muscles 104 which is in the trochanter (Fig. 7, $5B_2a$) and 110 (Fig. 9), and also all the sub-units of muscle 107, the depressor of the tibia. The arrangement of the separation of the fibres which supply m107 differs with the specimen ; however, each twig usually supplies three or four of the sub-units and sometimes the muscle units receive fibres from two branches of $5B_2$. There is also a sensory nerve which diverges from $5B_2$ in the base of the femur and which runs between the edges of the muscles 106 and 107 in the posterior femoral wall. After giving off branches to this region it terminates dorsally near the membrane between the femur and the tibia. In Fig. 9, this nerve has been cut short within a third of its length due to the complexity of the arrangement of the structures in this region of the femur.

The remaining fibres of $5B_1$ and $5B_2$ then continue into the tibia supplying the muscles which originate there and have their insertions in the base of the tarsus.

It was found that details of the arrangement of the units of the muscles in the femur were most easily investigated in a newly-moulded adult. The chitin is then fairly soft and pliable, therefore the attachments of the muscles were not so easily broken when deflecting the frontal wall of the femur as shown in Fig. 9. The skeleton of an older specimen is more brittle and small pieces are apt to break off when grasped by dissecting instruments.

Nerve 6 or Recurrent nerve

The sixth or recurrent nerve emerges from the posterior edge of the ganglion adjacent to the connective and runs posteriorly to join the first nerve of the metathoracic ganglion (Fig. 10). A branch, M.Rn.a, from the recurrent nerve, runs anteriorly, ventral to m93 and dorsal to the mesothoracic ganglion and it divides to innervate the muscles 87 and 88 (Fig. 2, M.Rn.a_1 and M.Rn.a_2).

Median nerve and some comparative notes

The median nerve emerges dorsally from the posterior part of the mesothoracic ganglion, dividing at the level of the anterior end of the second spina (Fig. 10), one branch going to each side of the segment. Each branch, con-

sisting of two axons (which can be seen clearly when stained by the methylene blue), runs close to the dorsal surface of muscle 93 nearly to the point of its insertion and then turns dorsally towards the spiracle, where it enters the spiracular muscle.

Since the spiracular nerve (spir. $n._2$) is joined by a branch of the first metathoracic nerve (Mt. 1), it is necessary, at this point, to consider the arrangement of the branches of Mt. 1 which occur in this region. The first branch of Mt. 1 runs anteriorly to the small muscle 116 (Fig. 10, Mt.1A) : the second branch

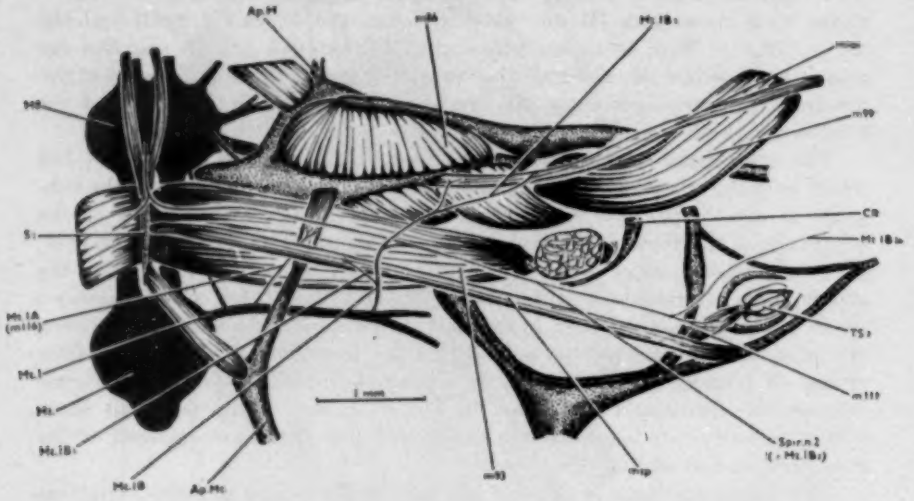

Fig. 10—A dorsal view of the mesothoracic ganglion and the nerve which innervates the second thoracic spiracle of the right side. (Key to abbreviations, p. 431)

(Mt.1B) also runs anteriorly, dividing twice so that there are three branches. There is in this area a fine muscle (msp) which is dorsal to 93 and which stretches from the second spina to the margin of the mesothoracic epimeron near the second thoracic or metathoracic spiracle (Ewer, 1954 a, b & c :—the mesothoracic spina-pleural muscle ; and Maki, 1938 :—anterior ventral transverse muscle, number 69). This muscle, which was found in newly-moulted adults, but which degenerates within ten to fourteen days leaving only a few strands of connective and fatty tissue, is innervated by $Mt.1B_1$ (N.B. Fig. 10 is drawn from a newly-moulted adult). The spiracular nerve (spir.$n._2$) runs parallel to this muscle and just beyond the point where the nerve crosses muscle 116 it is joined by $Mt.1B_2$. There are variations in the positions of the points of departure of these branches, $Mt.1B_1$ and $Mt.1B_2$, from Mt.1B. The final branch, $Mt.1B_2$ passes dorsally over the spiracular nerve and innervates another small muscle (mloi) ; Ewer (1954 a & b) refers to this muscle as the mesothoracic lateral oblique intersegmental muscle.

The combination of spir.$n._2$ and $Mt.1B_2$ continues toward the spiracle and a fine branch departs from it close to muscle 111 (closing muscle of second

spiracle). From dissection it would appear that this small branch comes from Mt.1B_2, therefore it has been labelled Mt.1B_2a in Fig. 10 : it turns dorsally and innervates a nymphal muscle (Table 1) to which Ewer (1954 a & b) gives the name mesothoracic pleural sub-alar muscle (Maki, 1938—number 48). The spiracular nerve (spir.n.$_2$) continues towards the spiracular muscle and it would appear from dissection that it is accompanied by the remainder of Mt.1B_2. Since these nerves are very small indeed, it is difficult to find the exact terminations of these branches.

From the evidence presented by dissection it is not improbable that the branch from Mt.1B_2 contains sensory fibres which supply the peritreme and associated integument of the spiracle. Hoyle (1959) found that the two axons from the median nerve are both motor, though evoking different heights of electrical and mechanical response and compared them with slow and fast fibres of the skeletal muscles. Also, he found no evidence for a peripherally-located sensory-motor nervous system affecting spiracle-function. However, it is probable that the cuticle of the peritreme has a sensory innervation ; it may therefore be appropriate to recall that the peritreme of the first thoracic spiracle is innervated by a sensory nerve which comes from the first nerve of the mesothoracic ganglion.

Ivanova (1956) states that the unpaired median nerve of the metathoracic ganglon of *Locusta migratoria* forms two nerve plexuses and that branches from these plexuses anastomose with the first, second and third pairs of somatic nerves (1, 3 and 5 of *Locusta* in this investigation ; i.e. those nerves which innervate the muscles of the legs and wings). No similar nerve plexuses were found to be associated with the mesothoracic median nerve in this investigation ; nor do any fibres of the branches of the median nerve pass into the nerves which supply the muscles of the legs and wings. Ivanova also mentions that the median nerve innervates a number of skeletal muscles which take part in the " rhythmic respiratory movements ". However, it is not clear from the report by Ivanova exactly which muscles are considered as being those which take part in the rhythmic respiratory movements. In this investigation, it was found that the branches of the mesothoracic median nerve innervate the muscles of the second thoracic spiracles and no evidence was found of any further ramifications of the median nerve.

Median nerve of prothoracic ganglion

The median nerve which leaves the dorsal surface of the posterior half of the prothoracic ganglion divides similarly and each branch travels towards the spiracles. The spiracular nerve of the first thoracic spiracle (Fig. 2, Spir.n.$_1$) is also joined by a branch of the first nerve of the ganglion immediately posterior to that which gives rise to the spiracular nerve : and in this case is joined by a branch of 1B of the mesothoracic ganglion. These nerves then supply the two spiracular muscles, 79 (closing muscle) and 80 (opening muscle) ; it would appear from dissection that both muscles are supplied by fibres from each nerve.

Muscle relationships

The arrangement and innervation of the spiracular, the intersegmental and the nymphal muscles, show interesting points of comparison. There is a temptation to suggest that muscle number 80 (opener of the first thoracic spiracle) at one time may have had its origin on the sternum, a position corresponding to that of the mesothoracic spina-pleural muscle. With this in mind it is worth noting that the opener muscles of the abdominal spiracles originate on lateral projections of the sternum. The reason for the presence of only one muscle (a closer) at the second thoracic spiracle may have some relation to the fact that the valves consist of thicker chitin than those of the other spiracles, and are so constructed that the spiracle is open when the closer muscle is relaxed.

Also, the intersegmental muscle number 59 may be homologous with the mesothoracic lateral oblique intersegmental muscle (mloi) which originates on the mesosternal apophysis. However, the insertions differ somewhat (Table 1) ; this, together with the fact that the mesothoracic muscle is very slender (degenerating as the cuticle hardens and the animal matures), may be because the mesothorax is separated by unsclerotised cuticle from the metathorax, only in the tergal region. Thus there is very little articulation between the metathorax and the mesothorax, whereas there is considerable movement between the prothorax and the mesothorax. Ewer (1954 c) has suggested that these nymphal muscles play a part in retaining body shape in the relatively soft-skinned nymph and in the newly-moulted adult. This seems likely, in view of the fact that most of the slender nymphal muscles degenerate as the adult matures.

Ganglionic connectives

Albrecht (1953) and Afify (1960) both figure median inter-ganglionic connectives in the thorax of *Locusta*. In this survey it has been found that the only direct connections between the prothoracic, mesothoracic and metathoracic ganglia are provided by the paired connectives ; there are no median unpaired nerves passing directly between any two thoracic ganglia.

DISCUSSION

It is interesting to compare these observations with those of others on similar insects. Many systems have been used to name the muscles and the nerves and it is thus difficult to make direct comparisons. It was thought, however, that an attempt at correlation might be aided by the construction of Table 1. This was based on the comparison of the nerves which innervate the homologous muscles of various species of insects from three insect orders. Although homologous muscles may vary in size and shape, due to the different proportions of the several insects, it was found that muscles could generally be identified under the names and numbers given by Snodgrass (1929). This identification made it possible to compare the nerves of each insect with those of *Locusta migratoria* and with one another.

Schmitt (1959) finds three pairs of lateral nerve roots from the mesothoracic ganglion of the grasshopper *Dissosteira carolina*. The first pair emerge from the

dorsal surface and are called the dorsal nerves (' DN ', Table 1). Also, there is a pair of fine dorsal nerves and a median dorsal nerve (' 3TN ') which makes a connection with the first metathoracic nerve (3DN). The arrangements of muscles in *Dissosteira* and *Locusta* are very similar, but it would appear that there are many differences in the nervous system. For instance, in *Locusta*, the muscles 60 and 61 are innervated by a branch of the prothoracic recurrent nerve P.Rn which joins the first mesothoracic nerve. In *Dissosteira* only muscle 60 is innervated in this way (by a branch of the nerve called 2ACn—" an anterior ganglionic connective " from the prothoracic ganglion to the dorsal nerve). The muscle number 61 is innervated by one of a pair of dorsal nerves from the prothoracic ganglion. In *Locusta* the muscles 87 and 88 are innervated by a branch of MRn, the mesothoracic recurrent nerve, which joins the first nerve of the metathoracic ganglion (Figs. 1 and 2). In *Dissosteira* muscle number 87 is supplied by a nerve which emerges from the main connective between the prothoracic ganglion and the mesothoracic ganglion ; 88 is supplied by a dorsal nerve from the mesothoracic ganglion. Thus the pattern which is found in *Locusta* with regard to the innervation of these ventral muscles is not easily recognizable in *Dissosteira*. Also, the connection between the transverse nerve (TN) and the first nerve (DN) is noted ; the muscle of the second thoracic spiracle is reported as being innervated by a branch of DN of the metathoracic ganglion and the transverse nerve TN of the mesothoracic ganglion. However, it is not clear whether the author finds a similar double innervation of the muscles of the first thoracic spiracle, although the innervation of these muscles by DN of the mesothoracic ganglion is reported.

The second nerve of *Dissosteira* (II) compares with 3 of *Locusta* and the third (III) compares with 4 and 5 (nerve III in illustrations by Schmitt (1959) is shown as having two branches close to its origin in the ganglion and in fact this may compare easily with the structure of 4 and 5 of *Locusta*). I have not been able to find a nerve in *Locusta* which corresponds to that which, in *Dissosteira*, innervates the aorta. This may be due to the fact that the dissection of the specimen involved cutting medially along the tergum to open out the thorax. Alexandrowicz (1926), reporting on *Periplaneta americana*, mentions that in the thorax the aorta is supplied by a branch of the nerve which supplies the dorsal muscles, and that this branch was not clearly identified since this region did not stain satisfactorily with the methylene blue.

Ewer (1954 a) has given a detailed account of the nervous system of the mesothorax of *Acanthacris ruficornis*, the tree locust. It deals with four paired nerves and the paired recurrent nerves of the prothoracic ganglion (r.n.I) and of the mesothoracic ganglion (r.n.II). The median unpaired nerves, Pro II of the prothoracic ganglion and Meso I of the mesothoracic ganglion are also reported. Nerves I and IV would seem to correspond fairly closely with 1 and 4 of *Locusta*. However, nerve II is called 3 in *Locusta* and III seems to correspond with 5. IID of *Acanthacris* is probably equivalent to 2 of *Locusta* as it innervates similar areas of the mesosternum ; there is no report of chordotonal organ with four groups of sense cells as in *Locusta*. The innerva-

Table 1—A comparison of the innervation of homologous muscles. This table has been constructed with reference Schmitt (1959) and Wittig (1955). The muscles are numbered according to Snodgrass (1929); those not included of the muscles those numbers which are enclosed in brackets refer to muscles which are not figured in any drawing. In other columns of nerves, those notes in brackets are additional details. In that column referring to *Dissosteira*, (i.e. not always the thoracic ganglion from which it arises). Similarly, in *Perla* and *Telea*, the thoracic segment is

Muscle	Number	Origin	Insertion	*Locusta*
Sterno-pleural intersegmental	59	upper end of prosternal apophysis	dorsal end of anterior margin of mesepisternum	$1B_2$
Second ventral longitudinal	60	prosternal apophysis	anterior rim of mesosternal apophysis	$P.Rn.a_1$
Sterno-spinal	61	base of prosternal apophysis	anterior end of first spina	$P.Rn.a_2$
First posterior rotator of prothoracic coxa	66	base of prosternal apophysis	posterior angle of coxa	(branch of prothoracic nerve 4)
Anterior rotator of prothoracic coxa (Ewer, 1955) (Maki, 1935 :—number 21)	66A	anterior to 66 on prosternal apophysis	anterior basal rim of coxa	(branch of prothoracic nerve 3)
Second posterior rotator of prothoracic coxa	67	first spina	posterior angle of coxa	(branch of prothoracic nerve 4)
Closing muscle of first thoracic spiracle	79	ventral projection of peritreme	lever projecting from posterior rim of spiracle	spir. $n_{-1}+1B_1$
Opening muscle of first thoracic spiracle	80	ventral projection of peritreme	base of posterior rim of spiracle	As 79
Longitudinal dorsal	81	first phragma	second phragma	$1D_1a$
Oblique dorsal	82	lateral part of scutum	outer part of middle phragma	$1D_1a$
First tergo-sternal	83	lateral prescutal lobe	mesosternum	$3A_1$
Second tergo-sternal	84	inner lateral scutalaria and posterior margin of prescutal lobe	mesosternum	$3A_1$
Pleuro-alar (wing flexor)	85	pleural ridge	ventral surface of third axillary	$4D_4$

to the works of Ewer (1954 a & b), Nijenhuis & Dresden (1952, 1955 a & b), Nüesch (1953, 1957), Pipa & Cook (1959), by Snodgrass are given either letters or numbers, or references to other authors. In the column listing the numbers In the column listing the nerves of *Locusta*, those nerves enclosed in brackets have not been included in the drawings. the arabic numeral placed before the letters ACn or TN indicates the thoracic segment in which the nerve travels indicated by roman numerals.

(Ewer) *Acanthacris*	(Schmitt) *Dissosteira*	(Nijenhuis & Dresden) and (Pipa & Cook) *Periplaneta*	(Wittig) *Perla*	(Nüesch) *Telea*
1ßb	DN (dorsal nerve)	2Br1a	IIN1	IIN1a
r.n.I	2ACn (proganglionic connective to DN)	2Ar1b (from " intercalary ")	IN8 (from connective)	IN4 + IIN2
r.n.I	nerve from dorsal surface of proganglion	2Ar1a (from " intercalary ")	IN8 (from connective)	IN3
IVCa				
IIєb				
IVCb				
1ßc + branch of posterior prothoracic unpaired nerve (Pro II)	DN	8 of proganglion + branch of 2B	IIN1 + " transverse nerve " (Ntr from INm)	ImN + branch of IIN1a
As 79	As 79	—	As 79	—
IBa	DN	2C	IIN1	IIN1b
IBα	DN	2C	IIN1	IIN1b
IIAa	II	—	IIN2	IIN2a
IIAa	II	—	IIN2	IIN3, IIN4
IVDb	III_1	3A	IIN2 + IIN7	IIN2a + IIN5c

[Continued next page]

[Table 1 continuation]

Muscle	Number	Origin	Insertion	*Locusta*
Pleuro-sternal	86	pleural apophysis	mesosternal apophysis	$4B_3$
Third ventral longitudinal	87	first spina	anterior edge mesosternal apophysis	$M.Rn.a_1$
Fourth ventral longitudinal	88	first spina	second spina	$M.Rn.a_2$
Tergal promotor of coxa	89	scutum	anterior angle of coxa near trochantin	$3A_4$
First tergal remotor	90	scutum	disc arising from posterior angle of coxa	$4D_1$
Second tergal remotor	91	outer end of ridge of scutum	posterior angle of coxa	$4D_2$
Coxa-spinal rudiment	(92A)	middle of first spina	anterior angle of coxa	$(3C_2a)$
Anterior rotator of coxa	92	sternellar ridge	anterior angle of coxa	$3C_1$
Posterior rotator of coxa	93	second spina	posterior angle of coxa	4C
Abductors of coxa	94	anterior ridge of episternum	anterior outer margin of coxa	$3A_2$
	95	anterior ridge of episternum	anterior outer margin of coxa	$3A_3$
	96	episternum posterior to 95	coxal membrane anterior to pleural articulation	$3A_2$
First pronator-extensor of forewing	97	first basalare	mesosternum	$3A_2$
Second pronator-extensor of forewing	98	first basalare	bases of apodemes of 94 and 95	$3A_3$
Depressor-extensor of wing	99	sub-alare plate of wing base	basicostal plate of meral region	$4D_3$
Adducators of coxa	100	posterior margin of mesosternal apophysis	posterior inner rim of coxa	$4B_1$
	101	posterior margin of mesosternal apophysis	posterior angle of coxa	$4B_2$
Levator of trochanter	102	dorsally, base of coxa	dorsal rim of base of trochanter	4A

(Ewer) *Acanthacris*	(Schmitt) *Dissosteira*	(Nijenhuis & Dresden) and (Pipa & Cook) *Periplaneta*	(Wittig) *Perla*	(Nüesch) *Telea*
IVBb	III_1	6B	IIN7	IIN5c
r.n.II	nerve from connective between pro- and mesoganglia	2Ara (metathorax) (+ 2Ar1b of mesothorax)	IIN8	IIN6
r.n.II	nerve from dorsal surface of mesoganglion	2Ar2 (metathorax)	—	—
IIAd	II	3A	IIN2	IIN4 IIN5e
IVDd	III	6A	IIN7	IIN5c
IVDc	III	4	IIN7	
$IICa_1$			IIN4	—
IICc	II	4	IIN4	IIN5a
IVC	III_1	6A	IIN7	IIN5d
$IIAc_2$	II	3A	—	—
$IIAc_3$	II	3A	—	—
$IIAc_1$	II	3A	IIN2	IIN2b
$IIAB_1$	II	—	IIN2	IIN2b
$IIAb_2$	II	—	IIN2	IIN2b
IVDa	III_1	—	IIN7	IIN5e
$IICb_2$	III_2	4	IIN4	—
IVBa	III_2	6B	IIN4	—
	III_2	6B	IIN3 IIN7	IIN5b+c

[Continued next page]

[Table 1 continuation]

Muscle	Number	Origin	Insertion	*Locusta*
Depressor of trochanter	103 a b c d	coxa (2 roots) margin of scutum lateral area of scutum ventral surface of meso-sternal apophysis	apodeme arising from ventral rim of trochanter	a $5A_2$ b, c } $3C_2$ d $3C_2$
Reduction of femur	104	base of trochanter	posterior rim of base of femur	$5B_2a$
Posterior levator (i.e. extensor of tibia)	106	base of trochanter	posterior rim of base of femur	$3B_2c+5B_1d$
Depressor (flexor) of tibia	107 a b c	 ventral proximal end of femur base of trochanter dorsal and ventral anterior wall of distal two-thirds of femur	apodeme arising from small plate in ventral membrane of knee-joint	$5B_2$
Retractor of claws	110	posterior wall of proximal end of femur (others in tibia)	plate at base of claws	$5B_2$
Closing muscle of second thoracic spiracle	111	lobe of posterior dorsal margin of meso-coxal cavity	ventral lobe of spiracular rim	spir.n_2 +Mt.$1B_2$
Mesothoracic spina-pleural (Ewer, 1954 a & b) (Maki, 1938, number 69)	msp	second spina, dorsal to 93	posterior edge of epimeron at spiracle	Mt.$1B_1$
Mesothoracic lateral oblique intersegmental (Ewer, 1954 a & b) (Maki, 1938 :—number 43)	mloi	posterior face of meso-sternal apophysis (near apex)	outer-facing edge of second phragma	Mt.$1B_2$
Mesothoracic pleural subalar (Ewer, 1954 a & b) (Maki, 1938 :—number 48)	( )	mesothoracic epimeron (lateral to 99)	cuticle near sub-alar sclerite	Mt.$1B_2a$
Fifth ventral longitudinal	116	posterior edge of meso-sternal apophysis	anterior edge of meta-sternal apophysis	Mt.1A

(Ewer) *Acanthacris*	(Schmitt) *Dissosteira*	(Nijenhuis & Dresden) and (Pipa & Cook) *Periplaneta*	(Wittig) *Perla*	(Nüesch) *Telea*
} IICa	a III_1 b } II c } d III_2	4 4 4 5	} IIN4	} IIN5b IIN5c+e IIN5d
IIIB	III_3	5 + 3B	IIN3 IIN7	
		3B	IIN6	IIN5b IIN5f
		5	IIN6	
			IIN6	
Meso IE	DN +3TN (from mesoganglion)	8 (mesoganglion) (+ 2Br1b ?)	branch of (IINtr + IIIN1)	IImN (which is joined to IIIN1)
Meso IF				
Meso IC			IIIN1b	
Meso IE			IIIN1b	IImN
IC (metathorax)	3Acn (mesoganglionic connective to 3DN of metaganglion)		IIN8	IIN6

tion of the muscles of the first thoracic spiracle agrees with that which is found in *Locusta* : however, the innervation of the second thoracic spiracle is notably different. Each lateral branch of the median nerve is described as having six branches. The first, Meso IA, joins r.n.II to run to muscles 87 and 88 ; there is no similar nervous connection in *Locusta*. The second branch Meso 1B, joins the nerve to the salivary gland. A connection of this type is also mentioned by Wittig (1955) in *Perla abdominalis*. In *Locusta* there is connective tissue associating the spiracular nerve with the nerve of the salivary gland in this region but no branch from the spiracular nerve actually joins the nerve of the salivary gland. The salivary gland and its nerve have many diffuse branches in this region and made dissection of the spiracular nerve quite difficult. The third branch, Meso IC, innervates the mesothoracic lateral oblique intersegmental muscle ; Meso ID joins the first metathoracic nerve. In *Locusta* it is a branch of the first metathoracic nerve which innervates the mesothoracic lateral oblique intersegmental muscle and another branch of it joins the spiracular nerve. In *Acanthacris* Meso IE supplies the spiracular muscle (N.B. it arises from ID after its connection with the metathoracic first nerve, although, indeed, it is stated that all the above muscles are supplied by the mesothoracic unpaired nerve). In *Locusta*, using intra vitam methylene blue injections, only two axons could be defined in the spiracular nerve until it was joined by the branch (Mt.$1B_2$) from the first metathoracic nerve. Therefore it seems unlikely that this nerve can send so many branches to these various destinations, namely to the recurrent nerve, to the salivary gland nerve, to the mesothoracic lateral oblique intersegmental muscle and also to the branch of the first metathoracic nerve.

Nijenhuis & Dresden (1952, 1955 a & b) and Pipa & Cook (1959) considered the anatomy of the nervous system of *Periplaneta americana* and noted eight pairs of nerves in association with the mesothoracic ganglion. The interganglionic connective is termed nerve 1 so that nerve 1 of *Locusta* is homologous with nerve 2 of *Periplaneta*. The nerve which is equivalent to the recurrent nerve of *Locusta* (P.Rn.) is considered to be a branch of the nerve 2 of the mesothoracic ganglion (2Ar2). This linkage (of PRn to nerve 1) in *Locusta*, is probably due to an interchange of fibres ; one group from the first mesothoracic nerve may join with fibres of the recurrent nerve to innervate muscles 60 and 61 ; another group of fibres from the recurrent nerve may join with the main part of nerve 1, probably with the branch 1D which goes to the muscles 81 and 82 (Fig. 8). This was demonstrated fairly well in trial dissections of *Schistocerca gregaria*, where the fibres of the recurrent nerve can be traced more easily since the interchanging fibres are separated and run independently of each other, the junction being triangular. This is supported by Nüesch (1954) in his work on *Telea polyphemus* where he found that some of the units of the dorsal longitudinal muscles did not develop if either the recurrent nerve or the first mesothoracic nerve was cut in the pupa. In *Periplaneta* the eighth nerve (i.e. the median dorsal nerve) of each of the prothoracic and of the mesothoracic ganglia innervates the spiracular muscles of the first and second thoracic spiracles respectively. Each is joined by two branches coming from nerve 2 of the ganglion posterior to it. However, the

authors (Pipa & Cook, 1959) are doubtful of the actual destinations of the two branches. Nijenhuis & Dresden (1952) found that the chordotonal organ of the femur of *Periplaneta* was innervated by a branch of 5 and 8 ; this compares with 5 B_1b and 5B_1c of *Locusta*. The same authors (1955 a & b) noted the muscle 104 (reductor of the femur) was innervated by two nerves from different roots (3B and 5). In *Locusta* it is the extensor of the tibia which is supplied by fibres from 3B and 5B. In *Periplaneta* the extensor of the tibia is innervated by 3B only. However, Pringle (1939), working on the metathoracic leg of this species, found a double innervation (i.e. " slow " and " fast " fibres) contained within the branch of 3B which supplies the extensor of the tibia. In *Locusta*, where the femur is only very slightly movable on the trochanter, the reductor of the femur is supplied by a small branch from 5B_2 (Fig. 7), but, as it is a very slight muscle in this species, it is not likely that its functions demand a double innervation of the type found in *Periplaneta*. Wittig (1955) also notes a double innervation of this muscle in *Perla abdominalis* (IIN3 and IIN7).

Wittig (1955) gives a detailed study of *Perla abdominalis* (larva and adult) ; there are eight pairs of nerves arising from the mesothoracic ganglion (the eighth being the equivalent of MRn of *Locusta*. There is also a median nerve (IINm) giving rise to the transverse nerves (IINtr) which, after joining with fibres from IIIN1b, innervates both muscles of the second thoracic spiracles (in *Perla* there are two spiracular muscles which operate the second thoracic spiracle). It is interesting to note that, in *Perla*, the nerves designated IIN2. IIN3 and IIN4 have a common root in the ganglion and that they compare in most parts with the three branches of nerve 3 of *Locusta*. Also, IIN5 of *Perla* is sensory and ventral to IIN2, IIN3 and IIN4 and is probably homologous with nerve 2 of *Locusta*.

Nüesch (1953, 1957), investigating the anatomy of the thorax of *Telea polyphemus* (Lepidoptera), listed six pairs of nerves in the mesothorax (the sixth connecting with the first nerve of the metathoracic ganglion as MRn does in *Locusta*). There is also a dorsal median nerve (IImN). Anatomical differences between *Telea* and *Locusta* are strongly emphasized, as many of the homologous muscles have innervations which are not easily comparable. For instance in *Locusta* m60 is supplied by a branch of the prothoracic recurrent nerve (Figs. 1 and 2), while in *Telea* the equivalent muscle is supplied by two nerves coming from different roots. One is termed IN4, which actually branches from the region where the connectives from the prothoracic ganglion join the mesothoracic ganglion : the other nerve which supplies this muscle is a branch of IIN2. Thus, the nerve which in *Locusta* travels in the nerve 1 and which joins the branch of PRn innervating m60 and m61, comes from the nerve IIN2 in Telea. Also, the muscle equivalent to m61 of *Locusta* is innervated by a nerve termed IN3 which emerges from the connectives near the prothoracic ganglion (a position corresponding to P.Rn. of *Locusta*).

Sensory nerves

A brief survey of the sensory nerves has been given in Table 2. This comparison is an approximation since it is more difficult to homologise the

various areas of integument than it is to compare definite muscles. It can be seen that in all species considered, the integument of the anterior sternum and the tergal region are supplied by the first nerve of the mesothoracic ganglion. In other areas the relationships are not obvious since the total numbers of nerves vary.

Table 2—A comparison of sensory innervation from work by Ewer (1954 a), Nijenhuis & Dresden (1952), Wittig (1955), Nüesch (1957) and Schmitt (1959)

Region	*Locusta*	(Ewer) *Acanthacris*	(Nijenhuis & Dresden) *Periplaneta*	(Wittig) *Perla*	(Nüesch) *Telea*	(Schmitt) *Dissosteira*
Sternum, spinasternum	1A	Iα			N1b	
Spiracle region, and intersegmental membrane	$1C_3$	IAα	2Br1d			
Costal region and tegulum	$1C_1$a	IAc, IAa				
Wing base	$1C_1$b	IAb	$2Br_3$	N1	N1c	DN
First phragma and surround	$1C_1$c					
Second phragma	$1D_1$b		2C			
Region of 4th axillary sclerite	$1D_2$	IBb	2Br2			
Sternum	2	IID	7	N5		
Episternum and insertion round 94 and 95	$3B_1$	IIBa	3Ar1a	N3+N7		
Region of trochantin and ventral coxal rim	$3B_3$	IIBb	3Br3	N3+N7		
Coxa	$3B_2$ (a & b)	(IIBa$_2$ to coxal cavity)	$3Br_2$	N4		
Ventral coxal rim near insertion of 92	$5A_1$	—	—	N7		
Femur and trochanter	branches of 5B	—	5r (5, 8 & 12)	—	N5f	

It is difficult to homologise the patterns of innervation of several authors, not only because there are different systems of nomenclature, but also because

the six species surveyed are from four insect orders and thus vary to some extent in their morphological characteristics.

SUMMARY

A. The anatomy of the nervous system of the mesothorax of the adult of *Locusta migratoria migratoroides* R. & F. can be summarized as follows :

1. There are six pairs of nerves issuing laterally from the mesothoracic ganglion. There is also a median nerve which emerges dorsally, divides immediately and sends one branch to the muscles of the second thoracic spiracle of each side. There is no median connective between any of the thoracic ganglia.
2. The second pair is probably wholly sensory, the fourth and sixth are probably wholly motor and the first, third and fifth contain both sensory and motor fibres.
3. The first pair of nerves of the mesothoracic ganglion has connections with a branch of the first nerve of the prothoracic ganglion and with the paired recurrent nerves and the median nerve of the prothoracic ganglion. It innervates the dorsal wing muscles of the first pair of wings and the intersegmental muscles : it also sends sensory branches to the sternum and to the tergum.
4. Several branches of the second pair contain fibres connected with the mesothoracic chordotonal organs which lie in each side of the mesosternum.
5. The third pair innervates the wing muscles and those coxal and trochanteral muscles which originate in that part of the mesothorax anterior to the pleural ridge. Also there is a fibre in each nerve of the third pair which joins with fibres of the fifth nerve in each trochanter of the mesothoracic legs and they innervate the muscles which extend the tibiae. The episternal region, the region of the membrane round the coxa and the coxa are supplied by sensory fibres from the third pair of nerves.
6. The muscles which originate on the pleural ridge and pleural arm, those wing muscles and coxal muscles which originate in the mesothorax posterior to the pleural ridge and the levator of the trochanter are supplied by the fourth pair of nerves.
7. The fifth pair innervates the intrinsic muscles of the second pair of legs and those units of the depressors of the trochanter which originate in the coxa. It also contains sensory fibres, most of which supply the integument of the femora and the femoral chordotonal organs.
8. The sixth pair or recurrent nerves join the first nerve of the metathoracic ganglion. Branches of the prothoracic and mesothoracic recurrent nerves supply the ventral longitudinal muscles.
9. The branches of the median nerve of the mesothoracic ganglion are each joined by a small nerve from the first metathoracic nerves and it may be that some part of the latter also contributes to the innervation of the second thoracic spiracle.

B.

1. Using the muscles and their homologues as a basis, an attempt has been made to compare the various systems of nomenclature as applied to the nerves. This has been summarized in tabular form.
2. With reference to these tables and to the works from which they were constructed, some outstanding features of the comparison of nerve distribution have been discussed.

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KEY TO THE ABBREVIATIONS USED IN THE DRAWINGS

Nervous System

P	Prothoracic ganglion	M.Rn.a2	Second branch of M.Rn.a
Mo	Mesothoracic ganglion	Mt.1A–B3	Branches of the first metathoracic nerve
Mt	Metathoracic ganglion	P.Rn.	Recurrent nerve from prothoracic ganglion
1A1–D2	First mesothoracic nerve and branches	P.Rn.a1	First branch of recurrent nerve from the prothoracic ganglion
2A1–C5	Second mesothoracic nerve and branches	P.Rn.a2	Second branch of recurrent nerve from the prothoracic ganglion
3A1–C3	Third mesothoracic nerve and branches	Spir.n.1	Nerve to the first thoracic spiracle
4A1–D4	Fourth mesothoracic nerve and branches	Spir.n.2	Nerve to second thoracic spiracle
5A1–B2	Fifth mesothoracic nerve and branches	M.Rn.a1	First branch of M.Rn.a
M.Rn.a	Branch of recurrent nerve from mesothoracic ganglion		

Skeleton and associated structures (except muscles)

Ap.M.	Mesosternal apophysis and projections	C	Coxa
Ap.Mt.	Projection of metasternal apophysis	CO	Femoral chordotonal organ
Ap.P.	Prosternal apophysis and projections	CR	Coxal rim
Ax.3	Third axillary sclerite	Cx	Branch of mesothoracic chordotonal organ to coxal rim
Ba	Branch of mesothoracic chordotonal organ to basisternum	Epm	Epimeron
Ba.S1	First basalare sclerite	Eps	Episternum
		F	Femur
		IM	Deflected intersegmental membrane

Mer	Meron
P.a	Pleural arm
P.r	Pleural ridge
S1	First spina
S2	Second spina
Sa	Sub-alar sclerite
So	Branch of mesothoracic chordotonal organ to region of first spina
Ss	Spinasternum
St	Branch of mesothoracic chordotonal organ to sternellum
Stl	Sternellum
T	Trochanter
Ti	Tibia
Tn	Trochantin
TS1	First thoracic spiracle
TS2	Second thoracic spiracle
103ap	Continuation of apodeme of muscle 103

POSTEMBRYONIC CHANGES IN THE REPRODUCTIVE SYSTEM OF THE SLUG *ARION ATER RUFUS* L.

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(With 8 figures in the text)

The morphology, histology and histochemistry of the reproductive system of *Arion ater rufus* (Gastropoda, Pulmonata) have been described. Contrary to previous opinion, the loop of the hermaphrodite duct has been found not to be a vesicula seminalis. Instead, the distal convoluted part of the duct acts as a true seminal vesicle. The changes which occur in the reproductive system during the life of the animal, have been examined in detail. Increasing age has a marked effect upon the development of the reproductive organs. It has been found that any of the epithelial cells of the acinar wall of ductules in the hermaphrodite gland may transform into a follicular cell. The nutritive function of nurse cells with relation to the development of spermatozoa is discussed. Fluctuations in the number of oocytes in the hermaphrodite gland have been determined and its significance in relation to the development of spermatozoa is discussed. *Arion ater rufus* is protandric. During the first month of life, each individual is male ; from two months onwards it is a true hermaphrodite, till finally it becomes female at the age between five to twelve months. Histological observations suggest that sex determination in the gametes is the result of an inherent capacity of the acinar epithelial cells to respond in different ways to an inducing mechanism. The distribution within the reproductive system of alkaline phosphatase, lipids, glycogen and other polysaccharides has been described. The significance of the distribution and quantitative changes of these materials is discussed.

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INTRODUCTION

Investigations on pulmonate molluscs are not numerous and much of it is in the older literature. The genus *Arion* itself was considered by Semper

(1857) who included in a general account of the anatomy and physiology of pulmonates, a description of the histology of its reproductive system. Related species were studied by Simroth (1883) who used *Agriolimax laevis*, Babor (1894), several species of Stylommatophora, Buresch (1911) on *Arianta arbustorum* and Hoffman (1921) on *Limax maximus*. More recently Laviolette (1950, 1951, 1954) has described germinal differentiation in *A. fuscus* and Duncan (1958) the anatomy and physiology of the reproductive system of *Physa fontinalis* L.

The genus, *Arion*, epitomizes many of the main features of the pulmonate molluscs and especially emphasises the problems of hermaphroditism. This paper, therefore, presents a description of the development, maturation and senescence of the reproductive system of *Arion ater rufus*, Arionidae, Pulmonata.

MATERIALS AND METHODS

Animals were collected from allotments surrounding the city of Sheffield and kept in large tanks in conditions approximating to their natural habitat. Abundant food was given, consisting of lettuce, cabbage, carrots, potatoes, oats and mushrooms. Sometimes dried lettuce with "Bemax" and sodium alginate food (without dried milk) was given, following Standen (1951).

To maintain the necessary humidity, sphagnum clusters were kept in the tanks and sprinkled with water, The death rate was low, but some cases of cannibalism were observed. Egg batches were collected and hatched in flower-pots filled with sphagnum and fallen leaves. Populations were kept separately and small monthly random samples (2–3) taken, weighed and the colour of the animal noted. Animals were killed and fixed simultaneously by injecting fixing fluids. Immediately afterwards the whole reproductive system was dissected out and put in fresh fixative. It was impossible to dissect out the reproductive organs in the early stage of development owing to their small size. Therefore, in these cases, the whole animal was immersed in fixative under vacuum to facilitate penetration.

A number of fixatives were used, including aqueous Bouin's fluid, Carnoy's and Susa's solutions, cold acetone and Baker's formol-calcium. Tissues were cleared in xylol, embedded in paraffin wax (M.P. 56° C.) and sectioned transversely at a thickness of 4–6μ. Serial sections were drawn for the reconstruction of whole structures.

The stains employed to obtain a general histological picture included Ehrlich's haematoxylin, with alcoholic eosin and Masson's trichrome and azan stains. For cytoplasmic structure and inclusions the chrome-haematoxylin-phloxine method (Gomori 1941) and the fuchsin-paraldehyde method (Gomori, 1950) were used with success.

Alkaline phosphatase was detected by a slight modification of Gomori's (1941) method. To distinguish between predeposited calcium phosphate, which also gives a positive reaction, and alkaline phosphatase, the sections were treated for thirty minutes in 0·1 Mol. sodium citrate-citric acid buffer at pH5·0 before putting into the glycerophosphate mixture. This dissolved the precipitate, but did not harm the enzyme. The sections were incubated at 37°C.

for two hours in the glycerophosphate mixture. Confirmation was obtained by using a second method, the azo-dye (Gomori, 1953).

The investigation of lipid materials was based mainly on the techniques of Cain (1950). Schultz's cholesterol test was applied after oxidation of the sections for three days in a 5 per cent iron alum solution. Some frozen sections were treated with digitonin to precipitate sterols (Lison, 1936), and examined under polarized light for the precipitated cholesterol digitonids. Detection of polysaccharides and muco-polysaccharides was based on the periodic acid—Schiff reaction (PAS) using Hotchkiss' method (Pearse, 1953). PAS positive material was identified as follows—(Pearse, 1953): glycogen-saliva test, hyaluronic acid—hyalase, mucopolysaccharides—metachromatic stains, acid mucapolysaccharides—Hale's test. Galactogen was tested according to Grainger (1952).

The volume of the hermaphrodite and small albumen glands were estimated from drawings made, with a camera lucida, of every fifth or twentieth section, depending on the size of the gland, from a series of histological sections. The areas of the drawings of the sections were found by use of an "Allbrit" Stanley disc planimeter. The volume was then calculated from the figures so obtained by substitution in the formula $V=Za \times tn$, where Za is the total area of the sections measured, in microns squared;

t is the thickness of the sections in microns;

n the interval at which the sections were taken.

Because of the difficulty of adequately sectioning large albumen glands, their volumes were estimated by displacement after immersion in water in a measuring cylinder.

The total number of oocytes (Y) was counted in every nth section throughout the gland; the expression $n \times Y$ then gives the total number of such oocyte slices in the entire gland. The true number of oocytes "N" was then calculated by multiplying nY by a factor t/D (where t=section thickness and D=mean diameter of the oocytes) to correct for the appearance of oocytes in two or more consecutive sections. The mean diameter was obtained by taking ten or more oocytes at random from each gland, tracing their areas, measuring them with a planimeter and calculating from formula: $D=2\sqrt{\frac{\text{mean sect. area} \times 3.}{2}}$

The number of acini was found in the same way, but the number of oocytes or acini per unit volume of gland was calculated by dividing numbers (N) obtained by the volume of the gland. These calculations were carried out on all animals up to six months of age, to cover all stages of gametogenesis and at random on some older animal groups to cover several differences occasioned by age.

The tissues for these estimations were all treated in comparable ways, that is fixed in Bouin's fluid and processed similarly, on the grounds that variations due to shrinkage would be of the same order.

GROSS ANATOMY OF THE REPRODUCTIVE SYSTEM

In *Arion ater rufus*, when fully developed (Fig. 1) the hermaphrodite gland or ovotestis (HG) is placed dorsally behind the stomach and between the lobes

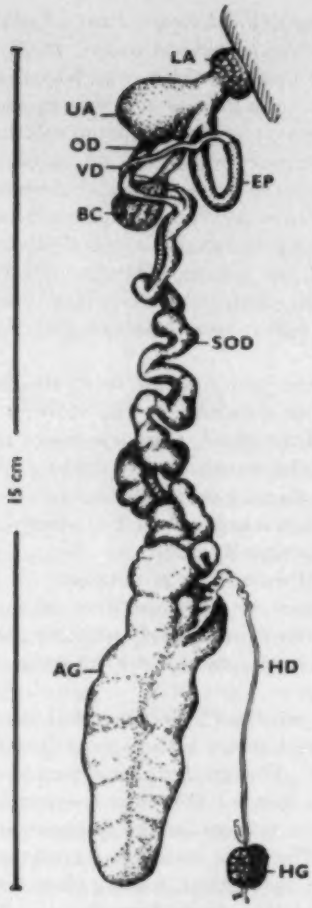

Fig. 1—Drawing of the reproductive system of a twelve month old *Arion ater rufus*.
(Key to lettering, p. 468)

of the digestive gland. The hermaphroditic duct (HD) emerges from the hermaphrodite gland as a conjoint canal which is straight proximally but convoluted distally. The duct, after making a sharp loop (Fig. 2) terminates in the spermoviduct (SOD). The distal loop sometimes called the " vesicula seminalis " is partly surrounded by the albumen gland (AG). The duct of the albumen gland joins the spermoviduct at the same level as the " vesicula seminalis ". The spermoviduct is very long and convoluted and consists of two continuous half-channels—the sperm channel and the oviduct. Anteriorly, the spermoviduct splits and gives two separate ducts—the vas deferens (VD) which expands into a thicker epiphallus (EP) and the free oviduct (OD). Both soon open into the upper atrium (UA). Into it, with a short stalk, also opens the globose spermatheca or bursa copulatrix (BC). The upper atrium

presumably is an expansion of the free oviduct and forms a bulge on the side of the oviduct. The atrium terminates in a narrower part, the lower atrium (LA) which is invested by a glandular pad.

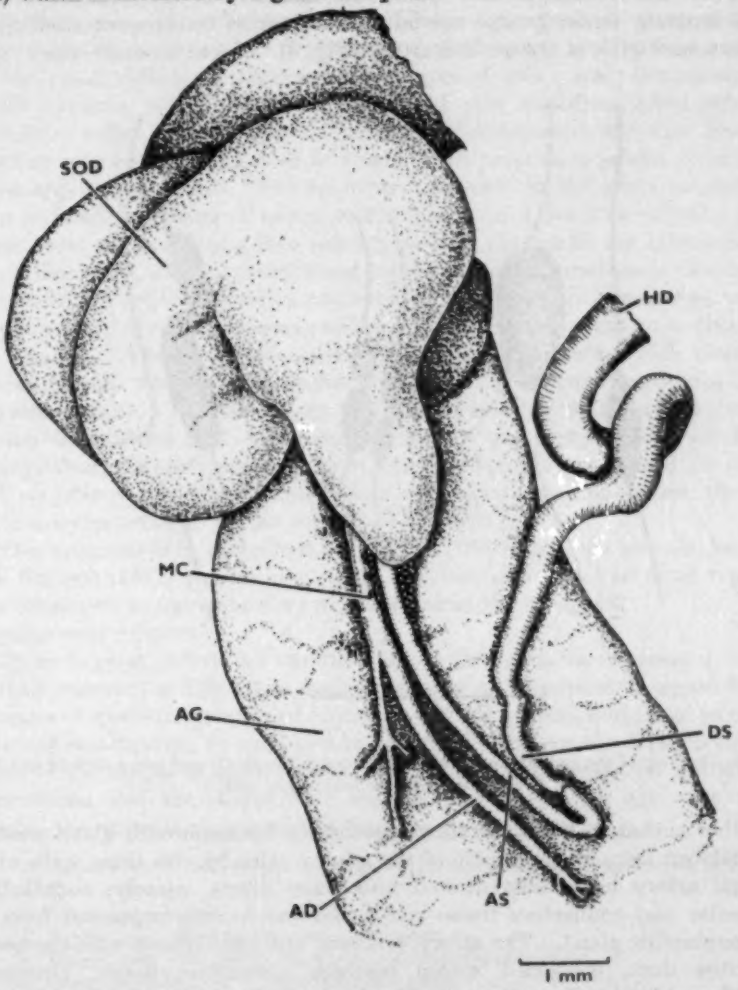

Fig. 2—Camera lucida drawing of a dissected albumen gland from a ten month old animal, showing the distal loop of the hermaphrodite duct. (Key to lettering, p. 468)

HISTOLOGY OF THE REPRODUCTIVE SYSTEM

The hermaphrodite gland

Development

The hermaphrodite gland, which is acinar in structure, comprizes two major lobes separated by the genital artery. Sections of newly-hatched *Arion a. rufus*

reveal several small groups of cells in the wall of the undifferentiated genital artery. These cells are the anlage of the primordial acini which are not yet fused to the hermaphrodite duct. In animals of one month of age, these separate acinar groups can be seen as minute transparent swellings on the external walls of the genital artery (Fig. 3). These separate anlage grow

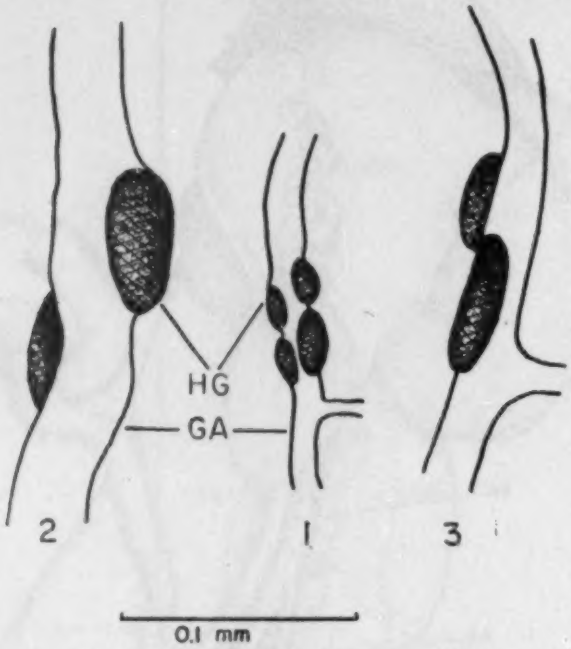

Fig. 3—Drawing of the hermaphrodite gland from a one month (1) and two month (2 and 3) old animals. (Key to lettering, p. 468)

together so that, at the age of three months, the hermaphrodite gland possesses the bilobed form characteristic of the adult. Also by this time, walls of the genital artery have differentiated into three layers, namely, endothelium, muscular and connective tissue layers and has become separated from the hermaphrodite gland. The artery, however, still runs closely with the hermaphrodite duct, contained within common connective tissue. Groups of undifferentiated cells remain at the base of the hermaphrodite duct. It is possible that these are capable of developing both into germinal epithelium and more acini. This suggestion is supported by the work of Laviolette (1951) who showed regeneration of acini around the hermaphrodite duct following castration.

Acini consist, in the first place, of two layers of cells, namely, an outer connective tissue layer (the tunica propria of Semper, 1857) and an inner epithelial layer, I shall refer to this latter layer as the acinar epithelium to distinguish it from the follicular epithelium. Between the acini there is always

a small amount of interacinar tissue which has not been mentioned by any of the previous workers. This tissue comprizes various cell types including mesenchymatous connective tissue, blood cells both singly and in groups, and nerve elements. The relative amount of interacinar tissue is greatest in the early stages of development, decreasing proportionately as the acini enlarge.

The acinar epithelium consists of four types of cells ; male spermatogonia, female oogonia, nurse (nutritive) cells and still undifferentiated germinal epithelium cells. During the first month of development, the four types of cells can only be found together in a very small proportion (about 5 per cent) of hermaphrodite glands. The sequence of events in the great majority of cases is firstly a change of acinar epithelium from a flat to a cuboidal form. There is no differentiation into cell types. All the nuclei are round (about 4·5μ in diameter), without nucleoli and with evenly distributed coarse chromatin. Many cells are actively dividing and slightly later stages of development show the products of division possessing nuclei of increased size (about 5μ in diameter) and nucleoli. These cells are spermatogonia. Those cells which have not divided remain without a nucleolus and cannot be differentiated into nurse cells and oogonia. For this reason, therefore, it is to be presumed that spermatogonia differentiate first. Furthermore, by the time the first oocytes can be distinguished, the spermatogonia have formed several layers around the acinar wall, sometimes filling the whole acinar cavity with, in some cases, the first spermatocytes evident in the middle of the acinus.

This sequence is in agreement with Ancel (1902) for *Helix pomatia* but not with Buresch (1911) and Richter (1935) who considered that all three types of cells developed at the same time at all stages of the life-cycle.

Gametogenesis—General

There is great individual variation in the development of *Arion a. rufus*. For this reason it is difficult to compare similar age groups with regard to the processes of spermatogenesis and oogenesis, though by examining large samples, a general classification by age could be made. In practice the detailed classification of the stages of gametogenesis was, of necessity, based on histological observations and the stages were roughly correlated with age. By these criteria, the animals examined fell into six groups ;

1. Animals which had reached the stage of spermatogonia and spermatocyte formation.
2. Spermatid stage.
3. Spermatozoa stage.
4. Oocyte stage, after discharge of spermatozoa.
5. Oocyte stage with partial atrophy of the hermaphrodite gland.
6. Complete atrophy of the hermaphrodite gland.

The body weights of animals in different age groups from one month to two years of age together with measurements of the hermaphrodite gland, its acinar content, the albumen gland at varying epochs in the waxing and waning of gametogenesis are brought together in Table 1. Table 2 shows the increase in volume of the acini, by proliferation of the acinar epithelium and the germ cells, as gametogenesis proceeds. The mean diameter, in microns, of the acini at the spermatocyte stage is 109·4 and this increases to 330·4 at the spermatid stage and 608·9 at the spermatozoa stage. After the discharge

Table 1—Gametogenesis at different ages of *Arion*

Age of animal	Stage of gametogenesis in hermaphrodite gland (based on histological observations)	Number of animals	Percentage of animals showing gametogenetic stage	Body weight (mg)	Volume of hermaphrodite gland (mm^3)
1 month	Spermatogonia-spermatoc.	22	100	305·1± 65·0	0·003± 0·001
2 months	Spermatogonia-spermatoc.	17	94	1064·0± 172·7	0·15 ± 0·01
	Spermatids		6	3416·7± 803·0	2·4 ± 1·6
3 months	Spermatogonia-spermatoc.	21	24	1972·2± 140·7	0·45 ± 0·03
	Spermatids		24	3569·8± 265·1	19·8 ± 6·4
	Spermatozoa		52	3950·0± 314·8	55·3 ±11·4
4 months	Spermatogonia-spermatoc.	17	18	3487·6± 361·4	1·3 ± 0·05
	Spermatids		18	4270·6± 462·8	7·5 ± 1·6
	Spermatozoa		64	6468·7± 921·0	53·1 ± 6·6
5 months	Spermatogonia-spermatoc.	17	25	6493·0± 719·0	2·0 ± 0·4
	Spermatids		6	5820·0± 400·3	15·8 ± 4·2
	Spermatozoa		46	9047·1±1478·0	38·8 ±14·9
	Oocytes		23	6000·0± 763·3	6·1 ± 0·2
6 months	Spermatids	19	11	9400·0±0	18·4 ± 0·00
	Spermatozoa		71	11562·5± 822·5	117·7 ±23·2
	Oocytes		18	6493·3± 918·7	3·2 ± 0·4
7 months	Spermatids	10	10	14750·0±0	7·5 ± 0·0
	Spermatozoa		60	10667·0±1600·0	110·7 ±45·6
	Oocytes		30	17400·0±1700·2	13·2 ± 3·0
8 months	Spermatids	17	17	12186·1± 572·2	30·8 ±10·5
	Spermatozoa		29	17285·6±1172·3	181·8 ±62·1
	Oocytes		54	14973·0±3047·2	13·0 ± 3·8
9 months	Spermatids	15	14	15361·2±2438·8	28·9 ± 3·5
	Spermatozoa		57	21442·6±1959·3	100·0± 22·8
	Oocytes		29	17057·8±5080·0	8·3 ± 1·6
10 months	Spermatozoa	16	70	20948·1±1542·0	190·4 ±11·6
	Oocytes		30	24917·6±3742·6	15·8 ± 4·4
11 months	Spermatozoa	8	40	26842·2±5142·3	132·7 ±13·2
	Oocytes		60	28900·7±3790·2	19·1 ± 4·5
12 months	Oocytes	9	100	25554·2±2737·0	34·1 ± 5·2
13 months	Oocytes and atrophy	6	100	20360·0±1630·0	23·0 ± 4·2
15 months	Oocytes and atrophy	3	100	20400·0± 860·4	17·6 ± 4·4
18 months	Oocytes and atrophy	2	100	24800·0± 720·6	9·6 ±0
2 years	Atrophy	2	100	73500·0±4000·0	9·2 ± 1·6

ater rufus. Figures are mean values ± standard errors

Volume of albumen gland (mm^3)	Diameter of acinus (in microns) (from formula given on p. 435)	Number of acini per gland	Number of acini per mm^3	Diameter of oocytes (in microns)	Number of oocytes per gland	Number of oocytes per mm^3
0·01 ± 0·001	100·3 ± 8·6	10·9 ± 2·4	3633·3 ± 419·2	—	—	—
0·04 ± 0·001	128·8 ± 7·5	188·9 ± 56·0	3774·2 ± 718·4	31·8 ± 2·5	258·9 ± 55·2	3615·3 ± 668·7
0·06 ± 0·001	147·2 ± 70·7	1131·5 ± 499·0	598·7 ± 99·5	46·4 ± 1·5	561·6 ± 293·0	263·5 ± 129·0
0·06 ± 0·02	53·5 ± 23·6	1998·7 ± 54·7	11792·0 ± 4180·0	46·0 ± 4·1	371·3 ± 71·4	2597·1 ± 254·7
0·33 ± 0·09	515·3 ± 81·3	566·3 ± 192·7	45·4 ± 13·0	57·3 ± 4·2	282·7 ± 87·6	19·0 ± 6·6
1·0 ± 0·1	563·7 ± 60·6	668·6 ± 84·2	14·6 ± 2·0	65·7 ± 3·0	435·7 ± 81·7	10·6 ± 2·0
0·08 ± 0·006	191·8 ± 46·6	749·7 ± 40·0	576·4 ± 21·1	48·4 ± 1·5	623·2 ± 25·1	687·2 ± 224·7
0·13 ± 0·01	301·9 ± 20·5	539·3 ± 59·9	76·6 ± 13·1	55·7 ± 1·6	572·8 ± 22·1	85·7 ± 23·0
97·3 ± 40·8	694·1 ± 49·3	702·9 ± 96·7	19·1 ± 4·7	77·3 ± 4·4	487·5 ± 26·8	11·2 ± 2·6
0·07 ± 0·007	175·2 ± 3·9	931·2 ± 185·0	577·0 ± 245·0	53·1 ± 5·3	703·7 ± 178·9	214·7 ± 59·3
23·0 ± 3·4	324·0 ± 70·6	1022·3 ± 203·1	67·3 ± 17·9	50·6 ± 8·1	776·2 ± 82·6	51·3 ± 6·6
274·2 ± 20·1	438·2 ± 79·6	705·5 ± 240·0	22·4 ± 3·2	98·4 ± 6·4	401·1 ± 60·9	19·6 ± 7·5
646·4 ± 124·0	466·0 ± 115·7	476·6 ± 210·0	80·6 ± 24·1	110·3 ± 7·4	659·0 ± 84·8	99·0 ± 21·4
0·2 ± 0·00	280·5 ± 40·4	2262·0 ± 734·0	127·5 ± 12·5	52·7 ± 2·8	860·5 ± 74·5	53·2 ± 11·5
2·7 ± 0·8	651·2 ± 60·0	895·4 ± 139·9	12·5 ± 2·7	78·4 ± 3·9	723·4 ± 76·2	9·0 ± 1·8
990·0 ± 98·8	286·3 ± 25·2	442·3 ± 85·7	138·6 ± 23·2	96·0 ± 12·1	1108·0 ± 222·1	361·6 ± 90·4
0·07 ± 0·0						
131·4 ± 22·0						
2917·7 ± 236·4						
1·4 ± 0·03						
255·1 ± 52·1						
2540·0 ± 103·7						
1·8 ± 1·0						
1401·3 ± 320·4						
2617·7 ± 741·2						
665·4 ± 346·0						
4452·3 ± 919·1						
3453·6 ± 706·1						
4340·0 ± 1104·0						
4137·5 ± 614·0						
2110·0 ± 400·6						
5200·0 ± 306·4						
7400·3 ± 602·1						
1606·6 ± 260·8						

of sperm, as would be expected, the acinar volumes decrease and at this stage their mean diameter is 392·3. During the oocyte stage there is no increase in acinar volume and it continues to decrease during the stage of atrophy. The collapse of the acinus after spermatogenesis occurs apparently because of the pressure from the rapidly proliferating inter-acinar connective tissue.

The number of acini is about ten per gland at one month old. This rapidly increases to a maximum in the spermatid stage (Table 2). At the spermatozoan stage at all ages, the number of acini is more or less constant and nearest to the true number of about 700 per gland. (Table 2.) The number of acini per unit volume (mm^3) drops as spermatogenesis progresses (Table 1).

Table 2—Mean number and diameter of the oocytes and acini in the hermaphrodite gland of *Arion ater rufus*, at various stages of gametogenesis

Stage of gametogenesis (Number of animals)	Number of oocytes per gland	Diameter of oocytes (μ)	Number of acini per gland	Diameter of acini (μ)
Spermatogonia-spermatocytes (42)	395·9 ± 58·6	43·0 ± 8·2	315·5 ± 114·8	109·4 ± 8·3
Spermatids (15)	527·2 ± 91·7	53·0 ± 1·8	951·8 ± 214·9	330·4 ± 47·1
Spermatozoa (40)	526·0 ± 44·3	77·6 ± 2·6	743·0 ± 62·3	608·9 ± 50·0
Oocytes (12)	963·8 ± 118·5	103·6 ± 5·9	748·6 ± 178·5	392·3 ± 30·0
Oocytes + atrophy (12)	346·3 ± 56·0	80·0 ± 16·3	*	*

* Values not counted because of the uncertain limits of acini at this stage.

Spermatogenesis begins soon after hatching and continues throughout the life of the animal until the oocyte or female stage of development of the hermaphrodite gland is reached. After differentiating from the acinar epithelium, spermatogonia do not move into the acinar cavity immediately, but remain connected with the wall of the acinus by a broader or narrower cytoplasmic stalk. These primary spermatogonia are then pushed away from the wall either by division of adjacent cells or by their own division, giving a second generation of spermatogonia. There is a continuous differentiation of new spermatogonia from the acinar epithelium, their proliferation continuing until the late phase of spermiogenesis. Not all spermatogonia, however, divide at the same time. In the early part of spermatogenesis several layers of spermatogonia belonging to both divisions can always be observed filling most of the acinar activity, before the first spermatocytes appear in the central part. In animals one month old, 10 per cent have reached this stage.

Spermatocytes have less cytoplasm but larger nuclei compared with spermatogonia. The primary spermatocyte stage seems to extend over a considerable period of time. They can be found in great numbers and of varying size and structure in the same acinus. The exact appearance of the secondary spermatocytes cannot be described, for they pass very quickly into the ensuing spermatocyte stage. Therefore it seems that the last spermatocyte stage is short and the meiotic division follows soon after the previous division. In animals aged two months, 6 per cent have reached the spermatid stage.

At this stage, the acini are densely packed with the developing germinal cells, which, by grouping around the nurse cells, begin their further maturation

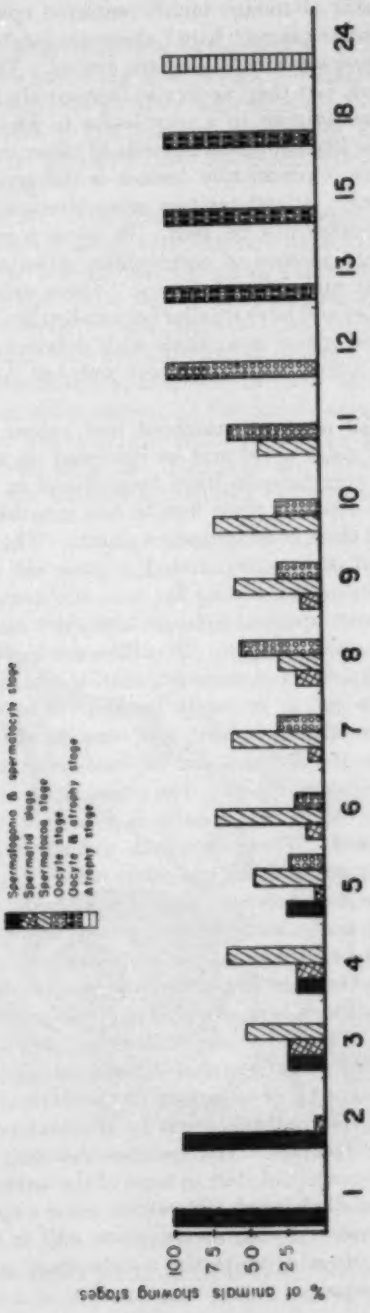

Fig. 4—Histogram showing changes, with time, in the distribution of sexual stages of *Arion a. rufus*.

immediately. There is still abundant undifferentiated epithelium, lining the acinar wall, although in some places "bare" stretches can be seen, where there is only the connective tissue layer bordering the acinus. The number of nurse cells has not been counted, but they appear to be most abundant at this stage.

There are always some animals in a population in which spermatogenesis has been delayed. In the hermaphrodite glands of these animals the relationship between somatic and reproductive tissues is different from those with earlier sexual development. Acinar cavities, more interacinar tissue and more and better developed oocytes can be seen. In some hermaphrodite glands calcium spherules or large patches of eosinophilic material can be observed in the cells of interacinar and arterial tissues. These animals with delayed spermatogenesis are smaller and have smaller hermaphrodite glands. Although the number of oocytes per gland in animals with delayed spermatogenesis is not higher than in the other (Table 1) their number per unit volume is greater.

At six months old, all animals examined had passed the spermatocyte stage, but the spermatid stage could still be observed up to nine months old (Fig. 4). The first ripe spermatozoa have been found in the acini of three months old animals, but animals from five to ten months of age have predominantly ripe sperm in their hermaphrodite glands. The maturing spermatozoa are usually grouped in bunches around a nurse cell or most commonly around its cytoplasmic strand extending far into the acinar cavity. Where a nurse cell is not present, spermatozoa are attached either to the acinar epithelium or to a degenerating oocyte. No difference in development can be observed between these gametes and those attached to the nurse cells, although it is evident that a nurse cell is normally present. The acinar wall at the spermatozoa stage is thin and expanded. It consists almost exclusively of the thin connective tissue theca which can be considered as a syncytium, for no cell boundaries can be distinguished. The nuclei scattered in it are flat and spindle-shaped ($2 \times 6{\cdot}5\mu$). Only occasionally is this layer doubled by patches of acinar epithelium cells. These residual acinar epithelial cells will subsequently develop into new oocytes and nurse cells. No further spermatogenesis takes place, since this process seems to occur only once during the animal's life. The timing and rate of spermatogenesis are not uniform throughout the entire gland. Spermatogenesis is less advanced in the peripherial acini, presumably because they are formed by later protrusion of the wall of the original acinus. There is also a lack of uniformity amongst the median acini, for while spermatozoa have been discharged in some, they are still ripening in others. No explanation for these regional differences can be offered. They are not related to the proximity of an artery or the hermaphrodite duct.

A certain quantity of spermatozoa needs to be developed before they are transferred to the acinar ductules. The spermatozoa stage can be observed in animals up to eleven months old, but in most of the animals at this age the the spermatozoa have been discharged. However, some animals up to thirteen months old have been observed with spermatozoa still in their acini. Here the delayed spermatozoa stage overlaps the oocyte stage and this problem is returned to in the Discussion.

Oogenesis

Oogenesis starts later than spermatogenesis. The oogonial stage has not been observed and the first stage of oogenesis is characterized by the appearance of oocytes. The young oocytes after differentiation from the acinar epithelium remain connected with it and become covered by the follicular membrane. This consists of several cells with their cytoplasm extended in a thin membrane over the whole oocyte.

The observations on *Arion* presented in this paper, show that the follicular membrane is formed from the adjacent acinar epithelial cells. If there is a nurse cell beside the oocyte, it sends a cytoplasmic extension to form only a part of the follicular membrane. The rest of the membrane is formed by any other cell adjacent to the oocyte. These could be called nurse cells, for histochemical observations show that they are nutritive in function, although they have not the histological appearance characteristic of nurse cells. This is supported by an observation on an older animal, at the oocyte stage, where several oocytes have developed at an abnormal site in the tubular part of the acinus among the ciliated epithelial cells (Fig. 5). Here the adjacent ciliated epithelial cells have made provisional follicles by sending cytoplasmic extensions round the

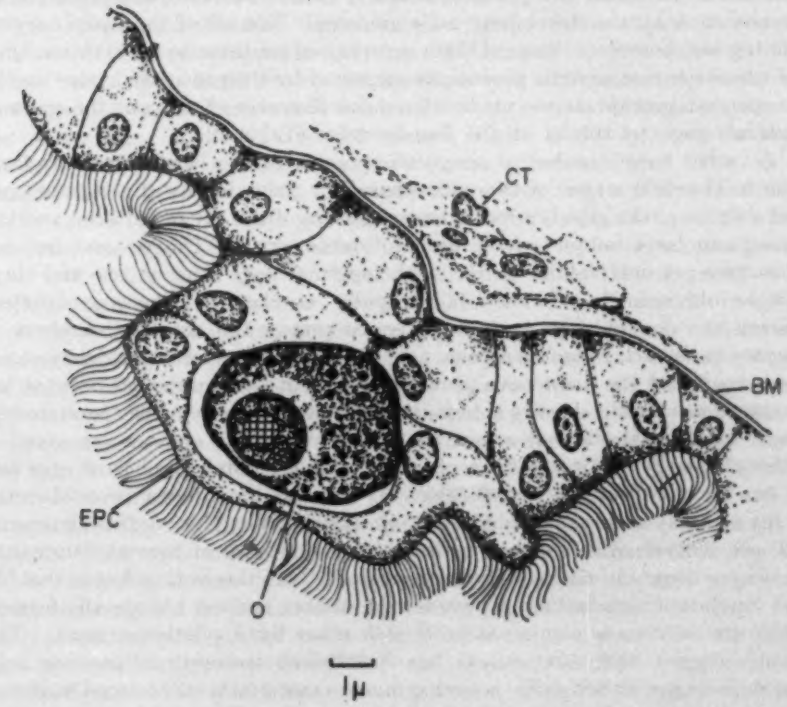

Fig. 5—Camera lucida drawing of part of the acinar ductule with an oocyte among the somatic, ciliated epithelial cells. (Key to lettering, p. 468)

oocytes. This indicates that even the ordinary somatic epithelial cells are potentially able to form a follicle. The follicular membrane covers the oocyte until the latter is released by rupture of the membrane. In one month old animals, only one out of the twenty-two examined has a single oocyte in its hermaphrodite gland. On the other hand, seventeen two-month old slugs, all have oocytes present in greatly increased numbers (mean number 395+58·6 at the spermatocyte stage. Table 2). In those animals two months of age still at the spermatocyte stage, the mean number of oocytes per gland is 260 but in those at the spermatid stage there are 561 oocytes per gland (Table 1).

During the spermatozoa stage, the number of oocytes in the age groups up to five months is more or less constant but it increases in the six months age group. Oogenesis is thus contemporary with spermatogenesis except for the first month. Oocytes of various sizes and at different stages of development are present at any time. No particular region of the acinus or part of the hermaphrodite gland is set aside for the development of oocytes ; they develop throughout the gland.

Most of the oocytes from the earlier stages of development do not reach maturity but disintegrate. This can be seen by changes in their cytological structure e.g. a darker staining reaction, the presence of coarse fibrous or vacuolized cytoplasm and pycnotic nuclei. Some of these disintegrating cells are absorbed by the developing male gametes. Not all of the early oocytes disintegrate, however ; some of them survive and continue to grow throughout the whole spermatogenetic process, as suggested by their increase in size during the spermatogenetic stages, viz. from a mean diameter of 43·0μ at the spermatogonial stage to 103·6μ at the female stage (Table 2).

A rather large number of oocytes is present in the hermaphrodite gland even in the final stages of the male phase but their density (i.e. number per unit volume of the gland) appears lower because of the expanded acini and the consequent large volume of the hermaphrodite gland. The greatest number of oocytes per unit volume is during the spermatocyte stage in two and three months old animals. It then falls rapidly as the acini become inflated. During the oocyte stage (complete female phase) the absolute numbers of oocytes in the acini reaches a peak. At this stage the spermatozoa have been discharged and the acini have grown smaller but the number of oocytes has increased so that the density is high, though not as high as in the spermatocyte stage. During the female stage, the mean number of oocytes per gland is 963·8 (Table 2) but it has been observed that the total number of eggs laid in two or more sessions is 200–300. My animals have never been observed to lay as many eggs, as recorded by Bronn (1933) although even this figure over 400 per individual is well below the potential number of eggs as determined by oocyte counts in the hermaphrodite glands. An interesting fact is that the last batches of eggs laid are always few in number and are abnormally formed. They are in strands connected with each other by a gelatinous mass. This would suggest that the animal has insufficient material to produce fully developed eggs, although the covering mucous material is still formed in abundance. It may be mentioned here that the oocytes when leaving the hermaphrodite gland have both yolky cytoplasm and the primary or vitelline

membrane. The latter can be seen as a darker staining line, surrounding the oocyte. Mucus is secreted by the spermoviduct. Thus, the abnormal eggs, described above, indicate some lack of correlation between secretions of the proximal and distal parts of the reproductive system.

Many of the oocytes remain in the hermaphrodite gland, becoming enclosed and absorbed by other tissues during the stage of atrophy. No division of oocytes during their development in the hermaphrodite gland has been observed Oogenesis in molluscs has been well described by Raven (1958) and in *Arion a. rufus* this process in general follows his description.

Vitellogenesis starts during the second month at the spermatocyte stage. The development of the nurse cells coincides with that of the oocytes. Both types of cell can be found in the same acinus in only about 6 per cent of the one month old animals, but they are present together in all the older ones. No time difference between the appearance of nurse cells and oocytes could be observed. Even if a short time difference exists, as Ancel has suggested in support of such a relationship between these two cell types, it is difficult to analyse, for not all the nurse cells develop at the same time, or in the same part of the acinus.

The nurse cells are small when first formed but possess all the characteristics of the large fully grown cells, such as the large coarsely granulated nucleus with deeply staining chromatin. This character easily differentiates the young cells from the developing male gametes. The size of the nucleus (ca $5 \times 11\mu$) of the nurse cell is small during the spermatocyte and spermatid stages, but increases as spermatogenesis proceeds until at the spermatozoa stage it measures about $10 \times 12\mu$. It is seen that the increase in size is mainly along the short axis of the original nucleus to a final almost spherical form. The cell is attached broadly at one end to the acinar wall, and extends well into the acinar cavity (up to 60μ). This cytoplasmic extension of the cell is the part to which the maturing sperm are attached in bunches. The cytoplasm becomes granulated and stains dark grey or black with fixatives containing osmic acid. With progressive spermiogenesis the nuclei of the nurse cells become irregular in shape, indicating a high metabolic activity in the cells. The most interesting phase of activity of the nurse cells begins at the late spermatozoa stage, and continues throughout the following stages. The nuclei of the nurse cells then become very irregular in shape, almost polymorphic and their chromatin is concentrated in larger lumps staining densely with haematoxylin or azan. The cytoplasm stains very feebly with eosin or azan and has a foamy appearance. The distal ends of the cells stretch as broad tongues into the acinar cavity, sometimes filling it completely (Fig. 6). Many of the still undifferentiated acinar cells join in this process. The hypertrophied nurse cells seem to have lost their normal function (feeding and supporting the male gametes). Those still engaged in feeding and protecting oocytes retain their shape and the chromatic density of the nuclei. Only after the release of the oocytes do these nurse cells hypertrophy like the rest. Ageing takes place. By increased proliferation of the interacinar connective tissue cells, the acinar cavities are obliterated and invaded by these cells. The nuclei of these hypertrophied nurse cells finally disintegrate until none are left in the acinus at the stage of

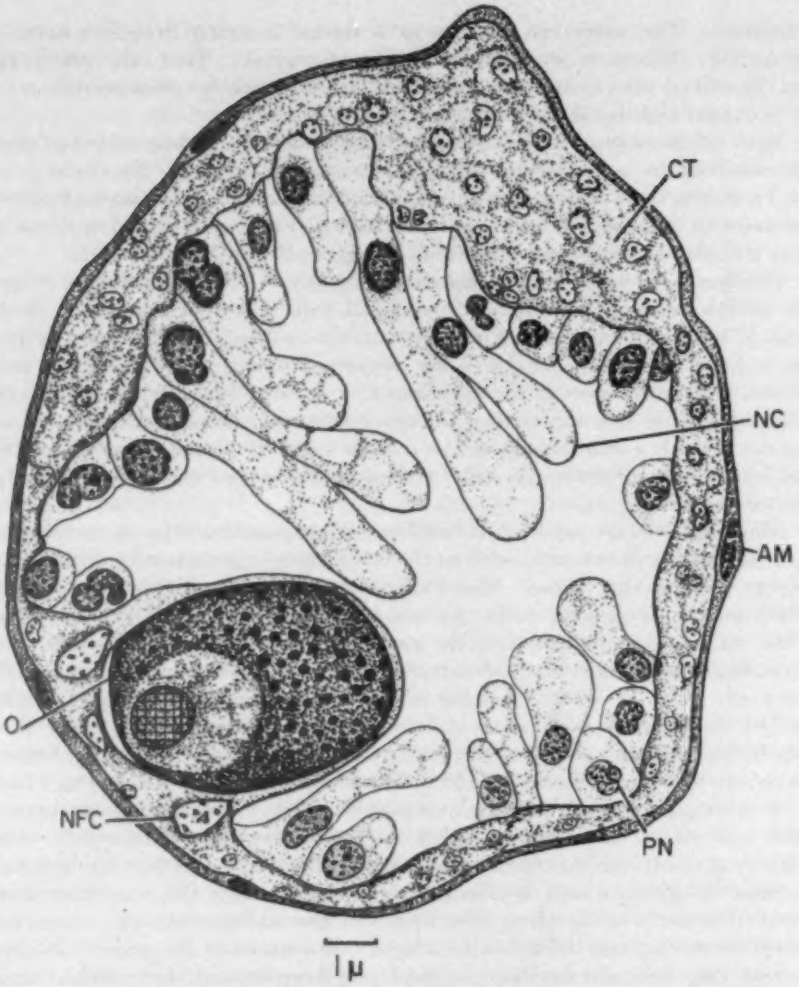

Fig. 6—Camera lucida drawing of an acinus which is undergoing atrophy, showing the hypertrophied nurse cells (NC) some of which contain polymorphic nuclei. An oocyte is still present, but the acinus is already invaded by connective tissue. (Key to lettering, p. 468)

atrophy. In the hermaphrodite gland of two year old animals it is hard to distinguish the former outlines of the acini. The whole gland is a mass of connective tissue stroma with islands of some deposit left by the nurse cells which are no longer distinguishable. Colour changes of the hermaphrodite gland follow the sexual stages by accumulation of more pigment in the connective tissues of the tunica and interacinar space.

To try to discover any correlation between the stages or development of the hermaphrodite gland and the accessory reproductive organs, the histological structure of the latter were also studied.

The hermaphrodite duct

The hermaphrodite duct, at its full development, consists of three layers of tissue. The inner layer is a ciliated cubical or columnar epithelium resting on a thin basement membrane. A thin connective tissue layer covers this inner one and a still thinner muscular layer comes between the two. At its earliest stage of development, as seen in transverse sections in a newly hatched animal, the hermaphrodite duct consists of a group of five radially arranged epithelial cells surrounding a small lumen situated beside the genital artery. The cells are smaller and darker stained than the cells of the acinar anlagen. Undifferentiated mesenchymal tissue cells surround both the artery and the duct.

In animals one month old, the hermaphrodite duct is formed of eight to fourteen cells, ciliated and columnar in shape (5–7μ high) radially arranged round the small lumen. The covering tissue layer which is not differentiated at this time into muscular and connective tissue layers is very thin at the level of the hermaphrodite gland, but increases in thickness further down. No coiling is observed in the distal region at this time.

In two and three month old animals (i.e. at the spermatocyte stage of development of the hermaphrodite gland) the number of duct-forming cells is increased to twenty to twenty-five and these are slightly larger (7–8μ) than in the newly hatched animals.

Great variation occurs in the width of the duct at different ages and different stages of sexual development. The development of the duct takes place regardless of any delay in spermatogenesis, so it may be assumed to be independent of the hermaphrodite gland development.

The lower part of the hermaphrodite duct shows the first signs of coiling at the spermatocyte stage. During the spermatozoa stage the hermaphrodite duct reaches its full development and becomes more coiled, at its lower or distal part. Longitudinal muscle fibres, which are probably responsible for driving sperm out of the coiled part at some later phase, may be seen here in the connective tissue mass outside the duct.

In many specimens, the hermaphrodite duct at the spermatozoa stage contains sperm along its whole length and the lower coiled part becomes expanded to a diameter of ca 800μ by the pressure of the accumulating sperm.

In some of the older animals (nine and ten months of age, the spermatozoa stage of development) changes in the epithelium are apparent. The nuclei become irregular in shape and the cytoplasm is vacuolated. After the emptying of acini, sperm is stored in the convoluted part of the duct until copulation.

During the oocyte state no radical change in the histological structure of the duct can be seen. In very old animals, after egg-laying is completed, the duct atrophies.

The most interesting part (Fig. 2) of the hermaphrodite duct is its distal end which is bent at a sharp angle to make a loop sometimes called the seminal vesicle (Pabst, 1914). Histological differences between this part and the rest of the hermaphrodite duct can be distinguished as early as the spermatocyte stage. The epithelium is here thicker and more folded. The muscle and connective tissue layers are thicker and some glandular cells are embedded

within them. At the spermatozoa stage, the differences become more obvious. While the convoluted part becomes wider, the "seminal vesicle" loop looks comparatively narrow because it seldom contains spermatozoa. Its high epithelial fold with long cilia almost fill the cavity and so prevent the sperm moving further down the tube. Sperm accumulate therefore in a dense mass just above the loop and seem not to be actively moving but to be carried passively by the cilia of the epithelium of the duct. This ciliated fold-barrier is probably opened at copulation by the pressure effected by the muscles along the duct or body wall. If this opening does not occur sperm accumulates also in the upper part of the hermaphrodite duct causing it to expand in a similar way to the convoluted portion.

The duct is different histologically above and below the loop. The first descending part is the direct straight continuation of the convoluted portion of the hermaphrodite duct which bends back towards the hermaphrodite gland. The second, ascending, part, leads towards the spermoviduct. Both sections are embedded in the tissues of the albumen gland. They have a folded epithelium with long cilia. The descending part, particularly near the bend, has deeper folds, yet is histologically still a part of the hermaphrodite duct. In older stages there is a vacuolated epithelium as in the convoluted part. On the other hand, the ascending part shows activity in its epithelium at this time. The cytoplasm is evenly stained with eosin, no vacuoles are present and the nuclei, which are uniformly elongated, have a finely granulated chromatin. At the bases of the epithelial folds and in the covering connective tissue layer there is a number of undifferentiated cells in the proximal region near the loop. They show only their large nuclei, without obvious cell membranes. More distally these cells demonstrate quite clearly their glandular nature and secretory activity. The ascending part can be looked upon as the beginning of the male part of the spermoviduct—the sperm-channel. Connected to the female or oviducal part the sperm-channel forms only half of the spermoviduct, but its histological structure is distinct along the length of the duct.

Albumen gland

The albumen gland is a pale-yellow, tubular organ, easily found in dissected mature animals, but rather inconspicuous in its early stages of development. As can be seen from Table 1 and Figs. 7, 8, development of the secretory epithelium of tubules in the albumen gland is slow and related to the increase in volume of the gland itself. The earliest stage, observed in sections of one month old slugs, is a tube-like formation lined on the inside with a neatly folded columnar epithelium (15–25μ). A thick layer of tissues consisting of undifferentiated cells cover the epithelium. The total diameter of the tube is 100μ. Following the gradual development of this blind-ended tube, which is at the end of the primordial spermoviduct it is possible to see the formation of side-tubes of the primary and secondary order, by proliferation and out-pushings of the epithelium. In this way a tubular system is formed, where the original tube becomes the central canal joined with its distal part to the spermoviduct. The side branches form the glandular units.

The histological structure of the albumen gland is similar to that in *Helix*

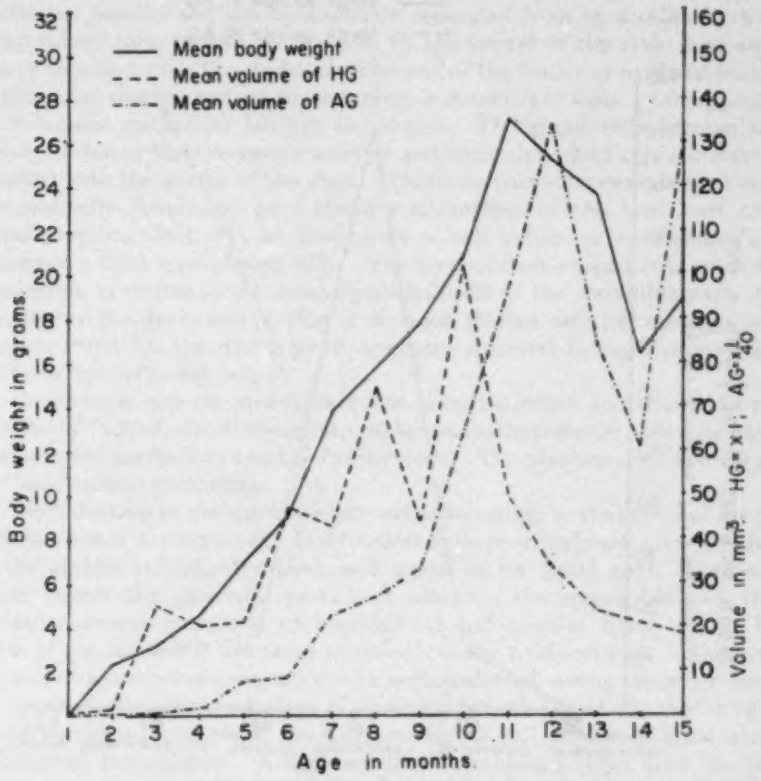

Fig. 7—Graph showing the mean body weight of *Arion a. rufus* compared with the mean volumes of the hermaphrodite and albumen glands at different ages.

pomatia and *Arianta arbustorum* (Krahelska, 1913). The histological differentiation of the tissues and the formation of glandular tubules continues through entire spermatocyte stage and well into the spermatozoa stage.

In the advanced spermatozoa stage the glandular cells become expanded and filled with secretory droplets. These turn into large globules at a later stage and stained with azan are blue in the red cytoplasmic framework.

An interesting phenomenon could be observed in the animals of ten and eleven months of age. Very often they were found dying with a protruding alimentary canal or lower part of the genital tract. These animals always had a hypertrophied albumen gland filling almost the whole body cavity; this appeared to be the reason for death. Most of these animals were still at the spermatozoa stage but some were at the oocyte stage. This suggests that these animals had not been able to control the secretion in the albumen gland. It was not possible to say whether hypersecretion was caused by delay in the oocyte stage in the absence of copulation or by not being able to stop secretion after laying eggs. Glandular tubules of these hypertrophied glands were

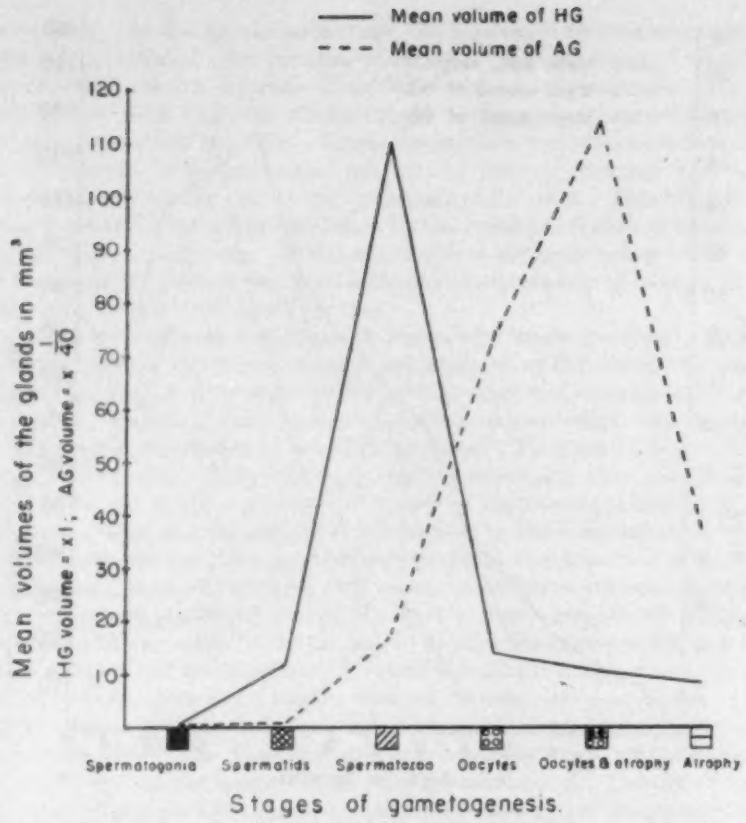

Fig. 8—Graph showing changes in volumes of the hermaphrodite and albumen glands in *Arion a. rufus* during various stages of gametogenesis.

expanded up to 300μ in diameter, and gland cells were packed with secretory material. Nuclei of these cells were irregularly shaped and deeply staining. Only some of the animals seem to recover from this hypertrophy. Such animals continue to grow but the albumen gland becomes smaller and finally atrophies ; its colour changes to dirty yellow. Only the surrounding connective tissue envelope reveals the previous dimensions of the gland. Histologically the tubular epithelium degenerates. In the gland of a two year old animal it is impossible to detect the form of the tubules. Deeply stained patches of secretory material are mixed with the great amount of intertubular connective tissues. Nuclei of the glandular cells, where they still can be seen, are small, surrounded by a thin layer of thickly granulated cytoplasm.

Spermoviduct

The spermoviduct, a thick, convoluted, glandular organ at its full development is compounded of two main parts ; the male and the female portion.

Both run parallel and are incompletely separated from each other by a deep longitudinal fold, formed on one side by the tissues of the male duct and on the other side by the female duct. The wall of the female or oviducal part is of a glandular mature and its inner surface is thrown into folds. Ciliary, cubical or columnar epithelium borders the lumen. The gland cells become much enlarged during their secretory activity and separately discharge the secretory product into the cavity of the duct. The male part or sperm-channel can be histologically considered as a direct continuation of the bent part of the hermaphrodite duct. It is lined with a tall columnar epithelium which possesses a thick coat of long cilia. The layer of flask-shaped cells around the epithelium is similar to the extra epithelial cells in the ascending part of the hermaphrodite duct, except that it is much thicker and its secretion better demonstrated by the discharge of secretory material through narrow ducts between the epithelial cells.

On its male side the spermoviduct is along its length accompanied by the " prostate " gland, which opens into the spermoviduct shortly before its separation into the spermduct and the free oviduct. The prostate duct is lined with a ciliary cubical epithelium.

The thickness of the spermoviduct varies according to the region of the duct and its stage of development. It is thickest at its proximal part, greatly reduced in the middle and slightly thickened again at its distal part. Connective tissue covers the glandular parts and also fills the spaces between them. Muscular tissues composed of longitudinal and circular fibres are in both parts of the duct, but are more abundant in the oviducal part. The artery lies close by and nerve elements can be seen scattered among the other tissues.

At its earliest observed stage of development, that is at one month of age in the spermatogonial stage, the spermoviduct is still undifferentiated into its histological components. A high columnar epithelium (10μ) lines the tube. No division into spermduct and free oviduct can be observed at this stage, although the bursa copulatrix has been formed as a tube and for a short distance runs parallel to the spermoviduct. A similar undeveloped histological picture can still be seen in animals two months old.

By the time they have reached three months of age the animals show much variation in the development of the spermoviduct. Those still at the spermatocyte stage do not show much differentiation of the duct into its different parts. However, the older the animal, the further the development has proceeded. In animals four months old, at the spermatocyte stage, the two parts of the spermoviduct have become more distinct due to the folded epithelium of the oviducal part and the accumulation of glandular cells along the male part. There is still, however, a thick layer of undifferentiated tissue round the epithelium.

In an animal at the age of five months but still at the spermatocyte stage, the spermoviduct is completely differentiated histologically. Prostate glandular units have been formed but do not yet secrete. Animal in which spermatogenesis is precociously advanced reach this stage of development at three months of age. A great number of cells can be seen in division in the tubular epithelium and in the tissues of the prostate gland. About 14–20

pyramid-like glandular epithelial cells (about $6{\cdot}5 \times 10\mu$ in size) form the tubular units in the protstate. There are also, as in the albumen gland, three to four intertubular cells bordering the inner side of the tubule. Eosinophilic droplets appear in the cytoplasmic space and nuclei are pushed back to the periphery, indicating that secretion has started.

The prostate gland in its secretory stage shows two differently with azan stained components in its glandular cells.

The epithelium of the sperm-channel is ciliated. The female part of the spermoviduct at an early spermatozoa stage is still very little differentiated. With increased age and the supervention of spermatogenesis all tissues concerned with the male part of the duct become fully developed. Epithelial cells become taller, up to 22μ in length and vacuoles and secretory droplets can be observed in some of them. At intervals, the epithelial layer is penetrated by the necks of the large flask-shaped extraepithelial glandular cells. These cells, while non-secretory have a large, round, darkly staining nucleus $7–8\mu$ in diameter surrounded by a thin corona of cytoplasm. The rest of the cytoplasm is dispersed in an irregular thin network. At the secretory phase a foamy basophilic secretory material fills the cytoplasmic cavity and is discharged into the lumen of the sperm-channel. While, during early spermatogenesis and particularly in younger animals, the oviducal part of the duct is still undifferentiated, in older animals, that have completed spermatogenesis, it begins to swell up and show secretory activity in its glandular part. The prostate is still secreting but is reduced in size.

At an early stage the secretion of the oviducal glandular cells is slightly basophilic, later it becomes intensely stained (probably it is more concentrated) and appears in the form of distinct droplets. The epithelium of the female part has short cilia and is secretory in nature. During oviposition maximal activity of the oviducal part of the spermoviduct can be observed. Its lumen is expanded and filled with secretory material. Eggs found in the middle part of the spermoviduct have already two or three layered secondary membranes. In the same animal eggs which have reached the lower atrium have four layered secondary membranes of which the outer one is darker than the three others. The sperm-channel does not stop secreting during oviposition. After discharging their secretory material parts of the oviducal glandular wall become vacuolated and the nuclei pycnotic.

Considerable changes take place in the spermoviduct of animals after twelve months of age, and particularly in the female part of the duct. Here the glandular coat is vacuolated and appears as large cysts of secretory material surrounded by invading connective tissue. In many cells these remains of the original secretory material are surrounded by a dark, granulated substance staining with chromhaematoxylin (Gomori). Some of the glandular cysts contain nothing but this dark granulated material. The epithelium is flat (7μ) and vacuolated, with nuclei measuring $1{\cdot}5 \times 5\mu$.

Vas deferens, the free oviduct and accessory structures

The spermoviduct separates into a shorter free oviduct and longer coiled sperm-duct and both pass to the upper atrium. This separation of the sperm-

oviduct into two complete ducts occurs during, or shortly after, the first month of the animal's life, and is always complete by two months of age. Soon after separation the oviduct has its walls formed of folded epithelium of varying thickness (6–30μ) and with a cover of undifferentiated cells.

At three months of age, regardless of the stage of spermatogenesis attained, both male and female ducts are differentiated from each other histologically and continue to grow. Shortly before entering the atrium the vas deferens forms the so-called epiphallus (Pilsbry, 1898) or the " Patronenstrecke " of the German authors (Pabst, 1914 and Simroth, 1887). It is merely enlarged and slightly modified distal part of the vas deferens which enters the atrium directly. Pabst (1914) believes that this part is responsible for the formation of spermatophores and replaces the flagellum present in other Pulmonates.

A penis is absent in *Arion*. The epiphallus has the same histological structure as the vas deferens but the epithelial folds are taller and in greater number. The ciliated epithelium also produces secretory material which can be observed as eosinophilic granules at the distal ends of the cells. Reticulate connective tissue composed of large spacious cells, with some muscle fibres penetrating amongst them, packs the extra-epithelial part. The vas deferens and the epiphallus are both surrounded by several layers of circular and longitudinal muscles. The muscular coat becomes much thicker just before the epiphallus enters the atrium. The amount of reticular connective tissues decreases, being replaced by muscle fibres. The epiphallus and the bursa copulatrix enter side by side into the right middle part of the atrium. No histological variation in the structure of the vas deferens or epiphallus have been observed during the male and female sexual phases. The vas deferens shows complete atrophy in an animal two years old. The epithelial layer is partly broken down and replaced by a mass of small connective tissue cells. These cells fill the cavity of the vas deferens and the intermuscular spaces.

Histologically, the free oviduct does not differ greatly from the sperm-duct. The glandular part present along the whole length of the spermoviduct disappears from the free oviduct. The duct is lined by a folded epithelium and covered with a thick layer of reticular connective tissue and muscular tissues. On entering the left-hand side of the atrium the oviduct is surrounded by a thick muscular fold which is formed by the atrial wall. This part is assumed to be vaginal. It continues the oviduct to the base of the lower atrium and separates the oviduct on its path through the lower atrium from that part of the atrium which receives the epiphallus and the bursa copulatrix. The bursa copulatrix, therefore, does not open into the oviduct but straight into the atrium.

The upper atrium is delimited by a wall lined inside with a folded, ciliated columnar epithelium. The epithelium is covered with a thick coat of longitudinal and circular muscle fibres. The fold which forms the vaginal part is also a muscular body lined with folded columnar epithelium. Bursa copulatrix appears in sections of one month old animals as a small tubular formation, derived from the upper atrium. In a transverse section the bursa appears as an oval body covered with a layer of connective tissue, and undifferentiated

cells with columnar epithelium lining the cavity. With increasing age and advancing sexual development the bursa copulatrix becomes differentiated, and assumes a flask shape with a narrow neck or stalk and a widened saccular part distally.

In animals three months old with spermatozoa in their hermaphrodite glands, the bursa copulatrix has reached its full histological development. In the stalk the ciliated epithelium bordering the cavity is deep (27–35μ) and folded. The nuclei of these epithelial cells are long ($5 \times 17\mu$) with coarse unevenly distributed chromatin and one or two nucleoli. The cytoplasm in the distal part of these cells is granulated, indicating secretory activity. The space outside the epithelial folds is occupied by reticular connective tissues with large cells resembling those seen round the arteries and the hermaphrodite duct. These connective tissues are penetrated by the muscle fibres extending inwards from the circular muscle coat which is three or four layered. An outer longitudinal muscle layer seems to form a part of the retractor muscle attached to the stalk of the bursa copulatrix. The saccular part of the bursa copulatrix is more expanded and contains some granular secretory material. The epithelium here is less folded and not so deep as in the stalk (20–30μ). The cytoplasmic structure of the epithelial cells is fibrous at their proximal end but granular and vacuolated at the distal end. Short cilia occur on a dense cytoplasmic layer. A thin connective tissue coat covers the epithelium and contains some muscle fibres.

No histological change or difference in activity has been observed between the spermatozoa and the oocyte stages. Only in the old animals of two years can symptoms of degeneration be seen.

The upper and lower atria

The earliest developmental stage of the upper and lower atria is observed in a newly-hatched animal when both atria are still parts of the undifferentiated primary reproductive tube. Their histological structure is exactly as Pabst (1914) has described it.

In animals of one month the widening of this tube indicates the origin of the upper atrium. No histological difference between the different parts of the widened tube can be observed. Even in animals two months old the histological differentiation is not much advanced. However, at this time, the wall of the tube opposite the bursa copulatrix shows small papillary folds of 20μ in transverse section. Such papillae can be seen on this side till the tube bends and turns towards the body wall. The epithelium lining the cavity varies in height between 10–20μ and is covered by a mass of connective and muscular tissues. During its passage through the body wall, the lower atrium is tightly restricted by the muscular tissues of the wall.

By the spermatozoa stage (three months old) both atria have undergone a rapid development, now being differentiated morphologically and histologically. The wall of the upper atrium is lined by a folded, ciliated cylindrical epithelium. Just behind the folds a number of cells with large irregularly shaped nuclei can be observed. The granulated and vacuolated cytoplasm of these cells suggests that they are of a glandular nature. A thick layer of

circular and longitudinal muscle fibres encloses the epithelium. The muscular fold of the wall, appearing in transverse section to occupy a central position, is also bordered by a folded columnar epithelium. Towards the lower atrium the cavity of the upper atrium becomes narrower but the thickness of the wall increases. The muscular wall becomes infiltrated by reticulate connective tissues, and saturated with glandular cells. These cells seem to show the greatest secretory activity of the time of copulation, however, they can be observed secreting in animals up to twelve months of age. In some animals, six and nine months old at the spermatozoa stage, the glandular cells have darkly stained oval nuclei ($10 \times 12\mu$) with finely granulated chromatin. The cytoplasm is slightly basophilic, vacuolated and some of the vacuoles contain secretory material which is non-metachromatically stained with toluidine blue. The nearer it approaches the body wall the more glandular the lower atrium becomes, till it finally fuses with the glandular epithelium of the skin. The folded epithelium can be seen to be penetrated by the ducts of extra-epithelial glandular cells and the foamy basophilic secretory material (blue with azan) can be seen discharging into the lower atrium.

Externally, this glandular part looks macroscopically like a whitish collar round the lower atrium ; it becomes everted during copulation and is sometimes protruded when the albumen gland hypertrophies. At an age when atrophy takes place in the other parts of the reproductive system, vacuolated glandular cells with pycnotic nuclei and large interstitial spaces filled with remnants of secretory material can be found in the lower atrium.

HISTOCHEMICAL OBSERVATIONS

The results obtained by the histochemical tests can be classified and compared according to the stage of sexual development the animals had reached. These stages are described on page 439.

Group 1—The spermatogonia and spermatocyte stage. Alkaline phosphatase activity appears in a zone around the acinus between the layers of spermatogonia and spermatocytes. No phosphatase positive reaction was obtained from the accessory reproductive organs.

The distribution of glycogen, which was granular in appearance, is similar to that for alkaline phosphatase. The hermaphrodite glands have a PAS positive zone between the spermatogonia and the spermatocytes, which does not appear in the saliva treated sections. Granules of glycogen occur in the acinar epithelium and the interacinar tissue, and a rather high concentration of glycogen is observed in the follicular epithelium and in the nurse cells. PAS positive material is found in the epithelial cells in the acinar ductules of the hermaphrodite gland and in the upper part of the hermaphrodite duct. After treatment of sections with saliva for thirty minutes the reaction to Schiff's reagent is negative, which indicates that this material contains glycogen.

Lipid droplets are concentrated in the acinar epithelium of the hermaphrodite gland and in the younger stages of the male germinal cells (spermatogonia). In some acini, sudanophilic droplets can be seen also in the cytoplasm of the spermatocytes. Lipids are present in oocytes but in more diffuse form, than in the spermatogonia. A rather large accumulation of coarse droplets can be

observed in the covering connective tissue of the acinus as well as in the interacinar tissue. Scattered spots stained with sudan black are found also in the arterial wall. No lipid material has been observed in the accessory part of the reproductive system.

Group II—The stage of spermatids and maturing sperm in the hermaphrodite gland. The nurse cells and the acinar epithelium show positive reaction for alkaline phosphatase. Spots of high activity in the interacinar tissues is observed. A positive reaction is also shown by the follicular epithelium, nucleoli of the oocytes, and the spermatocytes. In the hermaphrodite duct the brush-border or the ciliated part of the epithelium is positive for alkaline phosphatase along its whole length. The glycogen distribution is very similar to that of Group I.

A concentration of lipids in droplet form is found in the acinar epithelium and the spermatogonial layer. Scattered granules are present in the spermatocytes and nurse cells, but very few in the spermatids. Patches of accumulated lipid droplets occur also in the connective tissues, outside the hermaphrodite gland. In the oocytes, lipid material is concentrated around the nucleus. Treatment with acetone for twenty-four hours dissolves only part of the lipid material present, but pyridine dissolves it completely in the same time. In the accessory organs only the epithelium of the bursa copulatrix shows positive reaction to Sudan stains. The epithelial cells appear black with this stain due to the presence of minute black-stained droplets in the cytoplasm. A few black-stained droplets can also be seen in the glandular cells of the lower atrium.

Group III—Spermatozoa stage. In the hermaphrodite gland, alkaline phosphatase shows a similar distribution to that of the previous group.

In the hermaphrodite duct and some tubules of the albumen gland, the brush borders of the lining epithelium appear dark brown or black, indicating phosphatase activity. Positive reaction is also observed in some parts of the lower reproductive tract—the vas deferens, vaginal part of the free oviduct and the stalk of the bursa copulatrix. There is a particularly high concentration of glycogen in the nurse cells, so that their cytoplasm appears totally occupied by the granules. Scattered granules of glycogen are present in the acinar epithelium and in the gametes. Lipid material in droplet form is present in an increased amount in the cytoplasmic strands of the nurse cells. No quantitative change is observed in the sudanophilic material in oocytes, follicular epithelium nor any accessory reproductive organ. The glandular cells of the lower atrium show a great affinity for Sudan B and Sudan IV.

Group IV—The oocyte stage. Positive sites for alkaline phosphatase are the acinar epithelium, follicular epithelium and interacinar tissues, with the highest concentration in the acinar epithelium behind the hypertrophied nurse cells. Nucleoli of the oocytes and nurse cells also appear black. A slight positive reaction is also given by the brush-border of the free oviduct. Tests for alkaline phosphatase were not applied to the albumen gland at this stage because of the difficulties of cutting the acetone fixed glands. Most of the glycogen occurs in the cytoplasm of the nurse cells and in the acinar epithelium.

The glycogen granules can be seen accumulated in the follicular membrane and in the periphery of the oocyte. This may indicate the pathway whereby food reaches the oocyte. The hermaphrodite duct shows a marked amount of glycogen in its epithelium, particularly in its lower part.

Lipid material is increased in the cytoplasm of the nurse cells, and it is positive to Schultz's cholesterol test. The follicular epithelium shows a similar reaction to sudan stains and Schultz's test, and contains great amounts of lipids. Lipid droplets are more or less evenly distributed in the cytoplasm of the oocytes but are absent from the nucleus and nucleolus. They are present also in all parts of the hermaphrodite duct. None of these droplets in the oocytes and duct are positive to Schultz's test.

In the albumen gland at this stage, as in previous stages, small scattered droplets can be seen throughout the gland but no particular concentrations can be observed. In a few cases, more of the droplets seem to accumulate around the tubules of the gland and in the intertubular spaces.

Groups V and VI—Animals with atrophy of the reproductive organs. In the old animals, during atrophy of the reproductive organs only a slight positive reaction for alkaline phosphatase can be observed in the interacinar tissues of the hermaphrodite gland.

Small patches of glycogen are observed only in the interacinar tissues of the atrophic hermaphrodite glands. The pink PAS positive globules in the cytoplasm of the nurse cells is not glycogen. The albumen gland gives also a PAS positive reaction. Treatment of the sections with a liquid yeast culture at 37° C for six hours, and staining with toluidine blue and eosin, indicates that the secretory product of the albumen gland is galactogen. Apart from the hermaphrodite duct, glycogen is not detected in the accessory reproductive organs. Strong PAS positive reactions are noted but these were unchanged after treatment with saliva and are therefore considered to typify the presence of mucins. The secretion of the extraepithelial glandular cells of the sperm-channel stain a deep purplish with toluidine blue. The same reaction, although slightly paler, is given by the oviducal glandular part of the spermoviduct. The secretion of the prostate gland does not always give a positive reaction with PAS nor metachromasia with toluidine blue. The epithelial cells of the oviducal part of the spermoviduct and atria also produce a secretion which reacts positively with PAS and is slightly metachromatic with toluidine blue (pale purplish). The same reactions can be observed in the free oviduct and vas deferens. Here also, the cytoplasm of the reticulate connective tissues stains to a slight extent metachromatically with toluidine blue, suggesting that it is a mucin since mucins generally have a great affinity for basic dyes due presumably to their acidic nature.

Secretory material produced by the epithelial cells of the bursa copulatrix gives a positive PAS reaction, but only part of it stains metachromatically (β-metachromasia). Glandular cells of the lower atrium are PAS negative and non-metachromatic. Additional tests for mucins made with methylene blue at various pH and Hale's test did not help to identify the mucopolysaccharides any closer. Methylene blue was bound by all PAS positive tissues at pH 6·9

and pH 3·6. No distinct difference was observed between these two groups. The most intensively blue stained cells with Hale's test were the extra-epithelial cells of the spermoviduct, indicating the presence of acid-mucopolysaccharide.

In the hermaphrodite gland the acini have shrunk, and the small central cavity of each acinus is largely filled with sudanophilic material. Some of this material can still be identified as degenerating nurse cells but larger patches appear to be merely the fat remaining after the degeneration of groups of cells. The lipid content of the accessory reproductive system has increased. The oviducal part of the spermoviduct has lipid droplets in both the epithelial and glandular regions. Less sudanophil droplets are found in the epithelium of the sperm-channel but a considerable amount can be seen in the extraepithelial glandular cells and the prostate gland. Some isolated islands of lipid material in the glandular oviducal part of the spermoviduct gave positive results with Schultz's cholesterol test.

Calcium in the reproductive system

No particular tests for calcium deposits were made, but the controls used in testing for the presence of alkaline phosphatase were utilised to give information about the distribution of predeposited calcium. The presence of calcium spherules has also been observed in sections used for general histological examination.

In the hermaphrodite gland there are two main sites for the deposition of calcium, the wall of the artery and the interacinar tissues. A third site—the oocyte — which shows a predeposited phosphate compound is of a different nature, for here the deposit is not a by-product of metabolism, but an essential constituent of the yolk. The form and size of spherules differ in these two groups of tissues. Spherules in the arterial wall and in the interacinar tissues are assumed to be a deposited waste product, or a calcium reserve. These spherules of calcium with a darker centre begin to appear when the animal is two or three months old and can be observed in all stages of development. Smaller groups of spherules are present also in the accessory reproductive organs of older animals such as the bursa copulatrix, the hermaphrodite duct, the vas deferens, the oviduct and the atria. Larger amounts of calcium are deposited in animals with delayed spermatogenesis. The size of spherules varies but some of them reach 8μ in diameter.

DISCUSSION

Growth

Arion ater rufus, when kept under natural living conditions and given an adequate diet, continues to grow until the approach of death. This is shown graphically in Fig. 8, in which it is clearly demonstrated that change in weight is directly correlated with age. Death usually occurs at the age of eleven to twelve months and this probably represents the life span in nature. Death coincides with the end of the last sexual phase, that of the oocyte or female stage. However it is possible for some animals to survive for longer periods

when kept under optimum conditions and I had two individuals which reached the age of two years. Both these animals continued to grow until the terminal symptoms of death supervened.

The hermaphrodite gland reaches its largest size during the tenth month and then decreases when the spermatozoa are discharged and the oocyte phase is entered. The correlation between the body weight and the volumes of the hermaphrodite and albumen glands demonstrates that somatic and sexual development are in general synchronous. In detail, however, it was found that the initiation of the period of rapid growth of the albumen gland occurs later, at about four months of age, than that of the hermaphrodite gland. Moreover, the albumen gland continues to grow after the diminution in the size of the size of the hermaphrodite gland occurs. This finding is not surprising in view of the function of the albumen gland in supplying part of the material for the development of the eggs.

In many animals the continued growth of the albumen gland may lead to an almost pathological hypertrophy. This may constitute a significant factor causing the death of an animal. It was interesting to find that the two animals which survived to advanced old age had a small and atrophied albumen gland. Though the nature of the factors which condition hypertrophy and eventual atrophy are not known, it is clear that if an animal can overcome the period of hypertrophy of the albumen gland, the subsequent atrophy is beneficial for survival.

Hermaphroditism

The occurrence of sexual phases in *Arion* show that this animal is a protandric hermaphrodite (cf. Buresch, 1911 ; Pabst, 1914 ; Richter, 1935).

The protandry lasts for only the first month during which the animal shows the stages of spermatogonia and spermatocytes. From the age of two months onwards the hermaphrodite glands of all animals contain both male and female gametes and are therefore true hermaphrodites. The lengthy spermatozoa stage indicates both the prolonged development of sperm and its storage until favourable conditions may occur for copulation. The female phase is much shorter, as little as three to four weeks in some cases, and this explains the rarity of this condition in a random sample of a population. Later in life, by the age of twelve months, few animals have spermatozoa in the acini, and this stage may be regarded as a purely female one. At this stage oocytes are always present though in some cases oviposition may have taken place together with the onset of atrophy. Death normally ensues at the end of this female phase. In the rare cases of advanced old age sexual characteristics have disappeared.

The minimum time required to reach the stage of the formation of spermatozoa is three months (Fig. 4) but the animal is not fully mature at this age. Maturity occurs at the time when sperm have been discharged into the hermaphrodite duct. The interval of time between the former and the latter events is not known. It is known, however, that the discharge of sperm from the acini is a gradual process and that it is aided by the action of the cilia of the acinar ductules. Thus, in the early part of the spermatozoa stage, empty

acini may be found adjacent to those still full of sperm. It would follow that if sperm were ejaculated by musculare pressure, then there would be simultaneous discharge of all sperm.

In some individuals maturity may not be reached until nine months of age when only spermatids or maturing spermatozoa but no ripe sperm are to be found. No explanation can be offered for this great individual variation in the rate of development. And it is paradoxical to find that animals obtained from the same batch of eggs, kept under exactly the same conditions, develop at different rates. These differences may, of course, have a genetical origin.

Animals in which spermatogenesis is delayed are usually somatically less-developed and smaller in size. There is often an increase of tissue in the interacinar spaces together with an increased deposition of calcium and pigment. The relatively high proportion of oocytes in the hermaphrodite glands of these individuals suggests that the development of the male and female gametes is affected to different degrees. Oocytes seem to be less affected and to continue to develop whilst the normal development of male gametes is inhibited.

After the hermaphrodite gland has formed, there still remain undifferentiated cells around the genital artery and the hermaphrodite duct. These cells seem to act as a reserve for the replacement of tissues and probably possess the potentiality to produce new acini. The interacinar tissue itself has the role of supporting the gland but, in addition, food material such as glycogen and fat is stored there and it also accumulates waste products such as pigments and calcium. More of this tissue is present when spermatogenesis is delayed and in very old animals the amount increases so that it replaces most of the hermaphrodite gland. It is clear that the hermaphrodite gland arises from at least one anlage on each side of the genital artery and several may be seen at different levels. These fuse later to give the characteristic bilobed appearance of the gland. This clarifies the issue of the origin of this gland from primordial acini (cf. Pabst, 1914).

It has been observed that ordinary ciliated epithelial cells can be transformed into follicular epithelium and this finding suggests that they have been induced so to do by the oocytes. It would seem that any epithelial cell in the acinar wall or even in the duct may be transformed into a follicular cell. If this suggestion be correct it would resolve the difference of opinion between Ancel (1902) and Buresch (1911) concerning which cells give rise to the follicular membrane. My observations thus support Ancel's view that the male gametes start their development before that of the other two types of cell. It is difficult to resolve the problem of how the germinal cells become differentiated and what factors decide which of the acinar cells will give rise to male or female gametes. This problem is the crux of the phenomenon of hermaphroditism (Goldschmidt, 1923 ; 1931 ; Demoll, 1912 : White, 1945). Ancel (1902) considered that the nurse cells which appear immediately prior to the oocytes, may be such factors.

This would mean that the nurse cell induces oocyte formation in a closely adjacent " indifferent " cell which might otherwise develop into a male gamete. Therefore the evocator of femaleness (oocytes) would be the nurse cell. The fact that Buresch (1911) showed that nurse cells and oocytes appear simul-

taneously does not necessarily rule out the supposed inductive role of the nurse cell. However, my own observations serve to throw doubt on the Ancel-Buresch hypothesis. For in an acinus, the nurse cell is frequently in direct contact both with an oocyte and with a developing male gamete. It is difficult to maintain a hypothesis which supposes that the nurse cell induces femaleness in one adjacent cell yet fails to affect maleness in another.

Other observations have to be fitted into a general theory about hermaphroditism. Thus, there is a continuous appearance of new oocytes during the life of an animal after the nurse cells have been formed. Spermatozoa, too, may develop attached to the acinar epithelium or to a degenerate oocyte. Again, an oocyte may develop among the somatic epithelial cells in the acinar ductule *without* the presence of a nurse cell. Though it has not been possible to follow the development of such gametes in details, it would seem that they are less developed than those attached to nurse cells.

It seems likely that the main function of the nurse cells is, as their name implies, to nourish the developing gametes whether these be male or female. Those gametes which form away from nurse cells must needs be smaller than those produced adjacent to the nurse cells. The nurse cell, then, may be regarded as a link or transport unit between the acinus and the blood and supporting evidence comes from the presence of alkaline phosphatase and of glycogen.

If we rule out the possibility that nurse cells determine the sex of gametes, then other factors for the determination of sex must be sought.

Vitagliano (1950) in the case of the pteropod mollusc, *Cavolinia tridentata*, suggested that the determination of the sex of the germinal cells was caused by the spatial relationship of two kinds of follicle cells, one with little and one with abundant RNA. Although the cells in *Arion* were not tested for RNA content, the relationship between the acinar cells and the three types of cell developing from them, together with the absence of any particular spatial relationship, suggests that Vitagliano's hypothesis is not tenable in this species. In *Arion*, too, the fate of the cells is determined before they leave the one-layered acinar epithelium and male and female gametes develop side by side. There are no special zones in which the development of one or other of the types of cell takes place except that most of the male gametes, *after differentiation*, migrate into the lumen of the acinus. Here their further development occurs away from the acinar wall though they remain connected thereto by the extended cytoplasm of the nurse cell to which they are attached. This observation, indeed underlines the lack of influence of nurse cells on the determination of sex.

It seems unlikely that the interacinar tissue, the genital artery and the ductules of the acini, which comprize the somatic components of the gonads, are involved in sex determination. When the first spermatogonia appear, the interacinar tissue is still an aggregate of undifferentiated cells and, when the acini enlarge, it is widely scattered. Any hypothesis must explain how two adjacent cells in the acinar epithelium can develop simultaneously the one into a female and the other into a male gamete. It seems then that the determination of sex is not externally controlled. No external influence,

whatever its nature, could localize its action to the extent of differentiating between adjacent cells. However, if different cells possessed different capacities to respond to one and the same stimulus, then the outcome could be envisaged as male and as female gametes. Such inherent capacity to respond differently might be due to physiological or genetical factors or a combination of both. It is possible that there are differences in metabolic rate or varying concentrations of a determining substance.

There is no satisfactory explanation for the change in number of oocytes in the hermaphrodite gland during the different sexual stages. Pabst (1914) observed a decrease in the number of oocytes before copulation, that is at the spermatozoa stage. He suggested three possibilities to account for the decrement in oocyte number : (i) degeneration ; (ii) distribution to the acini ; or (iii) a combination of these two. However, my results show that the number of oocytes does not decrease as spermatogenesis proceeds in *Arion a. rufus*. In younger animals at the age of two or three months, the number of oocytes increases. In later age groups the relationship of the development of spermatozoa to that of oogenesis is difficult to express in absolute terms. It might appear, in the four to five month age groups, that there are, on the average, more oocytes at the spermatogonia than at the spermatozoa stage. Moreover, it is evident from the comparison of animals aged three to four months, that there is no significant difference in the number of oocytes when the acini enlarge. This view is supported by the fact that the number of oocytes per unit volume drops considerably as the volume of the hermaphrodite gland increases. The large increase in the number of oocytes during the oocyte stage is caused by the development of all the reserve cells in the acinar wall.

Accessory reproductive organs

The development of the accessory reproductive organs in normal animals is in step with that of the hermaphrodite gland. However, there is some indication that the stimulus for their development does not originate from the gland. In cases where spermatogenesis has been delayed, the accessory reproductive organs continue to develop at their normal rate, whilst the hermaphrodite gland exhibits a retarded development.

The hermaphrodite duct becomes coiled at its lower part to form a storage space for sperm and can be referred to as the " vesicula seminalis ". The loop of the duct at its distal end rarely contains sperm and even then in negligible amounts. The histological structure of this part of the duct shows that it is not suitable for storage or fertilization. Rather could it be, as Simroth (1883) suggested, a " Stauforrichtung ", or gathering arrangement which aids in the direction of the spermatozoa and eggs into the spermoviduct. This structure suggests a barrier or musculo-ciliary dam which serves to collect the sperm before copulation and effect its transport as a coherent mass through the sperm channel. This muscular loop is associated with a large number of nerve ganglia and probably facilitates the ejaculation of sperm into the sperm-channel. No data could be obtained about the later movements of sperm because only a few of the dissected animals had sperm in the sperm-channel. Pabst (1914) however, found the spermoviduct of the mature animal to be full of sperm.

Spermatozoa are confined to the sperm-channel in two ways. Long cilia, beating inwards, prevent the passage of sperm into the oviducal part and the semi-dividing fold acts as a mechanical barrier between the sperm-channel and the oviducal part. Eggs, on the other hand, are apparently not confined to the oviduct for, in some cases, eggs fill and expand both parts of the sperm-oviduct.

The extra-epithelial mucus gland was first figured by Pabst (1914) but he did not describe its structure or speculate on its function. The mucous secretion of this gland consists of an acid-mucopolysaccharide which seems to envelope the spermatozoa entering the sperm-channel. The secretion may well carry them to the distal part of the spermoviduct during copulation as it has staining reactions similar to those of the ground substance of the sperm-mass found in the bursa copulatrix.

The function of the prostate gland is obscure. Its position and the time at which it begins to secrete, suggest that it is a male accessory organ. The position of its opening at the distal end of the spermoviduct may indicate that this gland may take part in the copulatory process or in the formation of spermatophores. On the other hand the nature of its secretion suggests that it may be a source of nutritive material for the spermatozoa.

The oviducal part of the spermoviduct is responsible for the formation of the secondary egg membranes from the mucoprotein material which it secretes. Unless the spermatozoa are able to penetrate these membranes, fertilization must occur before the shell has been deposited and therefore occurs in the most proximal part of the spermoviduct. Deeper penetration of sperm through the loop and even into the hermaphrodite gland, as Buresch (1911) has suggested, is hardly conceivable. Self-fertilization cannot be excluded because of the anatomical measures, apparently arranged to prevent it, appear to be inadequate. On several occasions free oocytes have been observed while spermatozoa were present in the acini. Furthermore, in cases of delayed copulation eggs may be driven through the spermatozoa masses accumulated in the hermaphrodite duct. Since the lower part of the reproductive system is incompletely separated along its length, it would seem impossible to prevent the oocytes coming into contact with spermatozoa from the same individual.

The ageing process

Senescence is an important aspect of the reproductive system, but only Szabo (1935) has dealt with it previously, probably because of the difficulties in obtaining suitable material. Szabo, working on the histological correlation between the length of life and reproduction of *Agriolimax agrestis*, described "polimantis" cells in the hermaphrodite gland, clearly similar to the nurse cells of *Arion*. Szabo considered that as symptoms of atrophy and senescence were less obvious in those animals with well developed "polimantis" cells, these cells elaborated a substance lessening atrophy of organs and aiding in the prolongation of life. In *Arion*, however, hypertrophied nurse cells fill the acinar spaces by the end of the reproductive phase and are thus a consequence of the ageing process and do not inhibit it in any obvious way.

The reproductive organs undergo phases of activity and their cells differentiate, mature, function and then atrophy. Thus, a continuous process of destruction and neo-formation takes place in the hermaphrodite gland and in the accessory organs throughout the life of an animal. The acinar epithelium in the hermaphrodite gland is responsible for the production of new cells. Its productive capacity is limited. At the end of spermatogenesis, there are only a few areas of acinar epithelium left in the acinar wall. These are the reserves for the oocyte stage to come and they produce new oocytes and nurse cells. When this stage is finished, all the reserves are finally depleted and it is evident that the animal has completed its sexual life. No sexual stage can again be repeated because there is no acinar epithelium left. There is an innate limit to the life of the germinal epithelium ; it can be used up and it cannot be replenished but the factors involved are completely unknown.

The initial characteristics of senescence are best seen in the nurse cells and in the connective tissue cells of the theca and interacinar space. The lipid material which is already apparent in the nurse cells in small quantities in early spermatogenesis, increases considerably at the beginning of the female stage. It may be that this is a result of accumulation of surplus material rather than an increase in rate of production. The form of the lipid changes about this time from small droplets to large inclusions. Its steroidal nature was indicated by the Schultz and digitonin tests but there was no evidence of hormonal activity.

The nuclei of the nurse cells undergo a characteristic pyknosis. The nucleus becomes irregular and a process of budding turns it into a polymorphic or sometimes a mulberry-like body. The chromatin material stains more deeply and coalesces into large granules which finally form a single mass. The thecal and interacinar connective tissue proliferate and invade the acini. In animals at two years of age the amount of connective tissue exceeds that of the acinar remnants and it is filled with lipoidal substances. Lipids also accummulate in the accessory reproductive organs such as the spermoviduct, the prostate and albumen glands. In the spermoviduct some of this lipoidal substance is steroidal in nature and forms islands in the glandular oviducal part among the remains of its secretion. The vacuolated secretory parts of the female duct are surrounded by connective tissue stroma. Dark granules which appear in the tissue and stain with Gomori's chrome-haematoxylin, seem to be oxidized lipid. The accummulation of connective tissue in the accessory reproductive organs, and sometimes replacing the epithelium lining the ducts, is yet another symptom of ageing. The epithelial cells over a major part of the several ducts develop large vacuoles and pyknotic nuclei.

The more detailed information gained from the present work on the histological and histochemical structures of the different reproductive organs of *Arion a. rufus* will facilitate more exact study of the mechanism regulating the reproductive system. Since the development age and sexual stages have been compared, a basis has been established for an experimental study making use of biochemical and histochemical techniques.

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KEY TO THE LETTERING OF THE FIGURES

AD	Duct of the albumen gland	HD	Hermaphrodite duct
AG	Albumen gland	HG	Hermaphrodite gland
AM	Acinar membrane	LA	Lower atrium
AS	Ascending part of the loop	NC	Nurse cell
BC	Bursa copulatrix	NFC	Nucleus of a follicle cell
BM	Basal membrane	O	Oocyte
CT	Connective tissue	OD	Free oviduct
DS	Descending part of the loop	PN	Polymorphic nuclei of the nurse cells
EP	Epiphallus	SOD	Spermoviduct
EPC	Epithelial cells	UA	Upper atrium
GA	Genital artery	VD	Vas deferens

SOME OBSERVATIONS ON THE MEDIAL LOBULES OF THE EARS OF THE LONG-EARED BAT *PLECOTUS AURITUS* (LINNAEUS)

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Observations have been made on the changes occurring in the medial lobules of the ears of a Long-eared bat (*Plecotus auritus*) during the winter. These lobules became swollen and damp when the animal was torpid and shrivelled and a reddish colour when it awoke. The histological appearance of the lobule in its summer state is briefly described.

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INTRODUCTION

The ear lobules of the Long-eared bat *Plecotus auritus* (Linnaeus) (Pl. 1) are well known. They have been well described by Barrett-Hamilton and Hinton (1910–1921) in the following passage :—" Immediately above the point where the two ears meet, their margins form an angular notch ; above this, on each side, a small lobe projects laterally, so that when the ears are erect it touches its fellow ; these lobes are hairy, thicker, and more opaque than the rest of the ear."

The lobes are described or figured in a number of other studies of European mammals, among them Fatio (1869), Flower & Lydekker (1891), Millais (1904), and Rode & Didier (1946), and are shown in the better published photographs, e.g. that by Eric Hoskings (Pl. VII) in Harrison Matthews (1952). No account of changes in, or function of these lobules has apparently been published.

During the winter of 1959–1960 one of us, (W.G.T.), studying a captive bat, noted that the lobules varied in size and colour according to the level of the animal's activity ; later the lobule was examined microscopically in its summer or resting state (A.F.H.).

Our observations are presented here to draw attention to what we think may be an unrecorded aspect of bat biology, and in case further material becomes available elsewhere. They are incomplete without more prolonged study of the animal during the winter.

OBSERVATIONS ON AN ANIMAL IN CAPTIVITY

On 13th December 1959, a female Long-eared bat was taken from a dene-hole near Farningham, Kent, and has since been kept in captivity. (The animal was thriving in January 1961.) The bat was kept in a glass tank with a perforated zinc cover in an unheated room. It remained active intermittently throughout the winter, and when awake fed well on a diet of mealworms. It was regularly allowed to fly around the room.

Periods of activity and torpidity could not be correlated with fluctuations in room temperature. During one period the animal was inactive for nine days while the temperature remained below 50° F. (down to 42° F.), but it awoke to feed when the temperature rose to 52° F. Subsequently, however, it continued active with the temperature between 42° F. and 46° F., and on yet another occasion was torpid in a temperature of 52–56° F.

During these periods of fluctuation in activity it was found that the ear lobules varied in size and colour. When the animal was active they were shrivelled and dark red in colour. It looked as if they bore traces of coagulated blood, and when the condition was first noticed it was thought that the bat had injured itself. But the "injuries" were symmetrical; both lobes were equally affected. In this shrivelled, reddened state the appearance was quite unlike that exhibited in the summer when they are flesh-pink, thickened along the outer margins, and curved slightly inwards.

When the animal was cold to the touch and torpid the lobules were pale flesh coloured and considerably swollen. It was noted they exuded a colourless liquid, and this was apparently not due to condensation since the rest of the animal remained dry. The change from swollen to shrivelled could take place quite rapidly. In the evening of 12th March 1960, during a mild spell, the bat was handled while still torpid and exhibiting swollen, moisture exuding lobules. It awoke within a few minutes, accepted mealworms and groomed itself. Next morning the lobules had resumed their shrunken, reddened state; the animal continued to be active for the next three days. Observations made on 16th March suggested that the distension of the lobules preceded the onset of torpidity. After a period of three days' activity it was noted at midday that the structures were swollen and damp. When examined again eleven hours later the bat had lapsed once more into a state of suspended animation. Conversely the lobules showed a slight decrease in size on 18th March, and the following day when they were completely shrunken the bat awoke. On the 3rd April the bat was awakened while the lobules were enlarged and damp. It took several mealworms, then groomed its underparts. When examined 1½ hours later the lobules were shrivelled and dry. There were no further periods of torpidity and by the middle of April the lobules had assumed the appearance seen in good photographs, neither shrivelled nor swollen, but as slightly thicker projections of the medial edge of the pinnae.

The bat fed regularly until 29th October when it became rather sluggish, hanging still, with ears folded away, and taking no food for periods of four to seven days. It was examined during these periods of rest, usually without being touched and its eyes were half closed and the ear lobules unchanged. The animal occasionally stirred a little while being looked at, and could no doubt have been fully roused by a few moments handling.

OBSERVATIONS ON PRESERVED SPECIMENS

In an adult male bat from Knole Park, Sevenoaks, Kent, in late April, 1960, the lobule was not swollen. It measured 1·7 mm. in width by 2 mm. in length after being preserved in 70 per cent alcohol. The lobule was 2 mm. above the transverse flap of skin joining the two ears, it had a slightly shrivelled appearance, and was only a little more opaque than the rest of the ear. It was broadly club-shaped with a thin central region and thicker edges. The margin was folded over forwards a short distance from the edge and showed a series of short hairs. Close inspection revealed a plexus of small superficial blood vessels. Fixation in this specimen was too poor to allow histological examination.

In a second adult male specimen taken from a loft in Dunoon, Argyllshire, on 29th June 1960, the appearance was exactly the same as the above. The lobules were examined in stained serial sections. Each lobule contains a core of a thin, flat layer of elastic cartilage continuous with that of the pinna and covered with connective tissue. In section this cartilage has a Y-shaped outline, of which one limb (Pl. 2 A) continues above as the medial edge of the pinna. This limb is covered with a layer of adipose tissue approximately 0·3 mm. thick at the edge and tapering to the thin subcutaneous tissue of the pinna and the central part of the lobule. The other limb of cartilage (Pl. 2 B.) represents the body of the lobule and, at its edge, is covered by a pad of adipose tissue which, in this case, is folded inwards, and contains a number of hair follicles and their accompanying sebaceous glands. These follicles give rise to " unsegmented " sensory hairs (Pl. 2 C.). They contrast with the body hairs in which the arrangement of the outer scales gives a segmented appearance, (c.f. Harrison Matthews (1952) and Grassé (1955)).

The fatty tissue described above has no special features and is typical unilocular adipose tissue with numerous capillaries among the groups of cells. It is quite distinct from brown fat (multilocular adipose tissue) which forms the well-known " hibernating gland " found in other regions of the body in bats and other mammals. The irregular investing skin of the lobule has a thin keratinized epidermis similar to that of the rest of the ear. At the base there is a layer of striped muscle continuous with a layer at the base of the pinna. The blood supply of the lobule is from a fairly large vessel accompanied by a nerve running from the base of the pinna. It is worth noting that the lower fourth of the medial edge of the pinna has a very similar appearance in section to that described ; the edge of the thin cartilage of the external ear is covered with a pad of fat, with a few sensory hairs and a rich blood supply.

DISCUSSION

Examination of the specimens of *Plecotus auritus* in the British Museum of Natural History collection has shown that the lobules are a feature of all bats of the species at all times of the year. A photograph by Krause (Grassé, 1955) shows them to be present in an animal aged three days. They are not found in any other British bat, and we have not detected similar structures in drawings or photographs of other species. Dobson (1878) describes the ears of his species *Vesperugo* (syn. *Histiotus*) *velatus* as being similar in every way to those of *Plecotus auritus*, even possessing the lobule. *Histiotus velatus* is found in Brazil and should have no occasion to hibernate. The same author describes the species *Plecotus* (syn. *Corynorhinus*) *macrotis* as possessing a slight blunt projection in the position of the lobule in *P. auritus*. In his extensive paper on the hibernation of bats Eisentraut (1934) gives an illustration of a hibernating Long-eared bat but no changes in the lobules can be seen and no mention of such changes is made in the text.

Beyond recording these observations we have no explanation to offer. There was a definite relationship between the change in the lobules and the animal's periods of quiescence. The swelling preceded the suspension of activity and there were changes in the lobules after the swelling had subsided and the bat awakened. The changes were symmetrical in degree and in time, and this seemed to preclude a local pathological cause. The length of time they took to occur is possibly more compatible with a vascular than with a structural change. No help is obtained from the histological appearance of the lobule in its summer state and full conclusions must await more information about the animal during hibernation in other individuals, either in captivity or in the wild.

ACKNOWLEDGMENTS

We are indebted to :—Dr Gordon B. Corbett for examining the British Museum specimens ; to Mr R. W. Hayman of the Museum's mammal section ; to Mr P. C. Tinning for the Knole Park specimen ; to Mr K. M. Thompson for the Dunoon specimen ; to Professor G. M. Wyburn and to Dr K. M. Backhouse.

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EXPLANATION OF THE PLATES

Plate 1

The head and ears of a Long-eared bat with the ears partially folded back, the lobules discussed in the text are plainly visible in their summer state. (From a colour transparency by Keith Hyatt.)

Plate 2

A. Section through the medial border of the ear adjacent to the lobule.

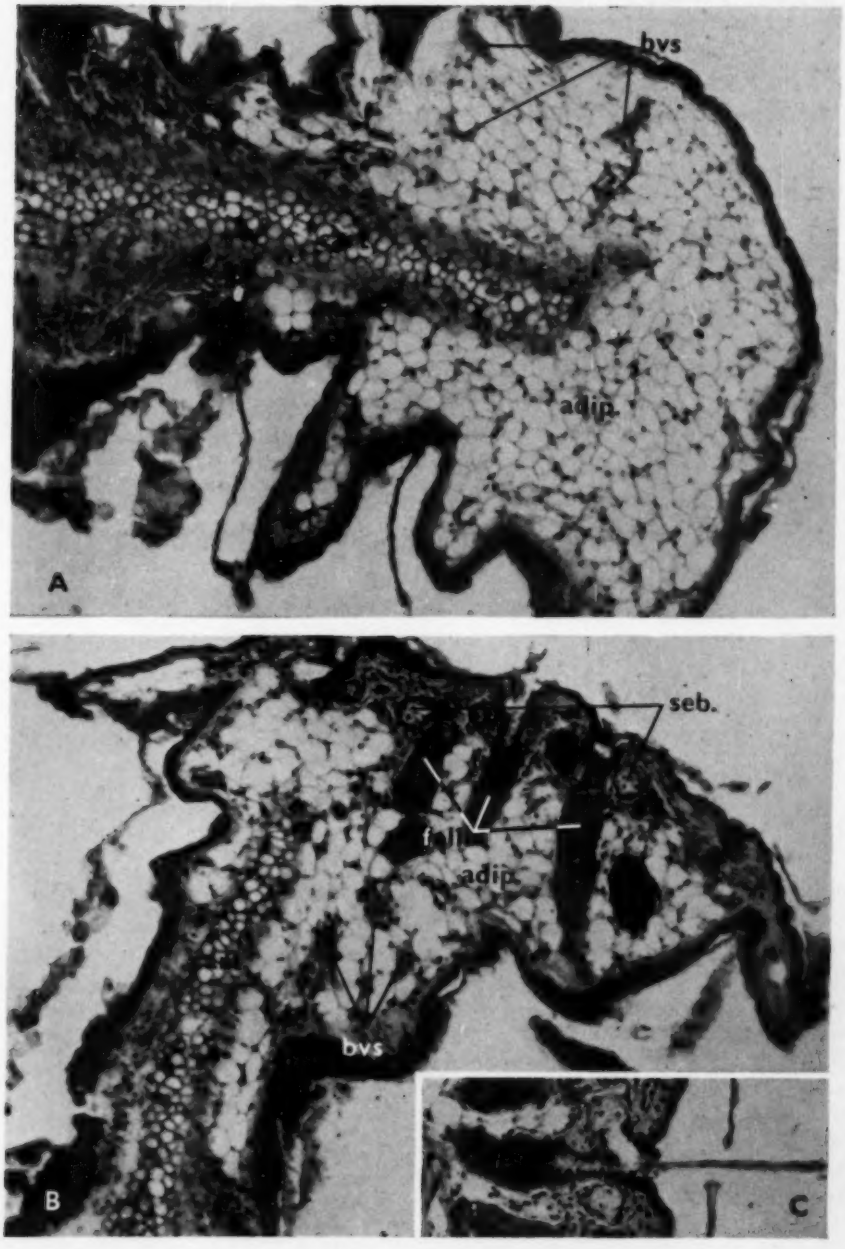
B. Section through the body of the lobule.

C. Hair follicle from the lobule, showing the " unsegmented " hairs found in this region (see text).

Abbreviations :

adip.	adipose tissue.
bvs.	blood vessels.
cart.	central elastic cartilage.
foll.	hair follicles.
seb.	sebaceous glands and their ducts.

The medial lobules of the ears of the Long-eared bat.



The medial lobules of the ears of the Long-eared bat.

THE COMPOSITION OF THE MILK OF THE AFRICAN BLACK RHINOCEROS (*DICEROS BICORNIS*; LINN.)

BY

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[Accepted 14th February 1961]

(With 1 figure in the text)

Little is known of the composition of the milk of the rhinoceros, partly because only so few have bred in captivity. We report here the chemical composition and vitamin content of a sample of milk of the African black rhinoceros (*Diceros bicornis*) taken during the nineteenth month of lactation.

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INTRODUCTION

The first African Black Rhinoceros born in captivity in this country was Roger, whose parents, Stephanie and Willie, arrived at the Bristol Zoo in October 1952 and were estimated to be then about eighteen months old. Roger was born on August 22nd 1958. When he was nineteen months old he was sold to the Chester Zoo. At this time he was still suckling, such a long suckling period being quite normal in this species. After he had been separated from his mother, the keeper noticed that Stephanie's udder was very tense and milk was being ejected from the teats. He was able to milk her without undue difficulty. For two weeks she gave daily about two quarts of milk, and then the yield began to decrease until, after a further two weeks, she was dry. As soon as we learnt that a rhinoceros at Bristol Zoo was lactating, we arranged with Mr R. E. Greed, the Director, to have some of the milk sent to Shinfield for analysis.

DIET

Stephanie's daily food intake was :

Cattle grazing nuts	4 lb
Flaked maize	1½ lb
Kale (winter) or cabbage or other green stuffs (summer)	8 lb
Clover or lucerne hay	76 lb
Mangolds or carrots	56 lb
Elm or evergreen oak branches *ad libitum*	

In addition, once a week, one-eighth of a pint of cod-liver oil was offered with the cattle grazing nuts, and also 4 lb linseed cake, 1½ lb bran and 1½ lb crushed oats.

MILK SAMPLES

About 300 ml of milk were collected each time on the second and third day after the removal of the youngster. The samples were stored in Polythene bottles and deep frozen as quickly as possible after collection. On arrival at Shinfield, the two samples were thawed and well mixed together, when portions were taken for the different analytical procedures. For the microbiological estimation of vitamins it was necessary to freeze the samples again until the assays could be done, but all other tests were done immediately on the thawed milk. The milk was white and very watery in appearance.

ANALYTICAL METHODS

The methods used for chemical measurement of the major constituents and for the microbiological assays of the B-vitamins in the milk were as described by Gregory, Kon, Rowland & Thompson (1955). Iron was measured by the method of Woiwod (1947). Riboflavin and thiamine were also measured fluorimetrically by the methods of Bessey, Lowry & Love (1949) and Houston, Kon & Thompson (1940). Total ascorbic acid was measured by the method of Roe & Kuether (1943) modified as recommended by Geschwind, Williams & Li (1951). The procedure described by Aschaffenburg & Drewry (1957) was used for the paper electrophoretic examination of the soluble proteins.

RESULTS AND DISCUSSION

1. *Chemical analysis*

The contents of the major constituents in the milk are shown in Table 1. One of the main differences between rhinoceros's and cow's milk is the low fat content of the former. Grzimek (1957) found an equally low content (0·27 per cent) in the milk of a rhinoceros at the Frankfurt Zoo. The stage of lactation of this rhinoceros was not stated but, like Stephanie, it was probably at mid or late lactation. An analysis of colostrum or early lactation milk would probably show a higher fat content, otherwise the young rhinoceros would receive very small quantities of fat soluble vitamins.

Table 1—The constituents other than vitamins of rhinoceros's milk.

	g per 100 *g*
Fat	Trace
Solids-not-fat	8·10
Lactose	6·06
Protein (Total N × 6·38)	1·54
Casein (N × 6·38)	1·11
Soluble proteins (N × 6·38)	0·29
Non-protein N	0·02
Ash	0·34
Calcium	0·056
Phosphorus	0·040
Sodium	0·037
Potassium	0·086
Chloride	0·079
	mg per 100 *g*
Iron	0·14

Grzimek's values for non-fatty solids (9·5 per cent), carbohydrates (7·2 per cent), casein (1·58 per cent) and ash (0·37 per cent) are similar to ours. In addition to being lower in fat, the rhinoceros's milk contained less protein and calcium than cow's milk, but the lactose content was higher.

The soluble protein/casein ratio was about the same as that normally found in cow's milk. Paper electrophoresis resolved the soluble protein fraction into at least five discernible components (Fig. 1a). The fraction was thus complex in composition like that of the milk of the cow (shown for comparison in Fig. 1b), sheep and sow, and unlike that of the group of species in which only a single major non-casein constituent is found, e.g. the goat and guinea-pig. From the mobilities it appears that both globulins and albumins are present.

2. *Vitamin content*

The vitamin content of the milk is shown in Table 2. The milk was similar to cow's milk in its content of ascorbic acid, calcium pantothenate and vitamin B_{12}. Values for nicotinic acid, biotin, riboflavin and vitamin B_6 were all less than those typical of cow's milk. Both microbiological and fluorimetric

Table 2—The vitamin content of rhinoceros's milk (Expressed as μg/ml milk).

Chemical assays	
Fat soluble vitamins	not measurable
Riboflavin free	0·44
total	0·58
Thiamine free	0·07
total	0·85
Ascorbic acid total	17
Microbiological assays	
Biotin	0·005
Nicotinic acid	0·09
Ca pantothenate	3·40
Riboflavin	0·15
Thiamine	0·73
Vitamin B_6 (pyridoxal)	0·04
Vitamin B_{12}	0·005

methods confirmed the high thiamine content of the milk. Fluorimetric tests showed that 90 per cent of the total thiamine activity was in a bound form, and the paper chromatographic method of Gregory (1960) demonstrated that the bound form comprised considerable quantities of thiamine monophosphate and some thiamine pyrophosphate and that, in addition, free thiamine was present. In cow's milk, phosphorylated thiamine is found only during the early stages of lactation. Our sample of rhinoceros's milk (taken at the nineteenth month of lactation) would probably correspond to the mid or late lactation secretion of the cow. Houston, Kon & Thompson (1940) have shown that in cow's and goat's milk, the content of phosphorylated thiamine is inversely proportional to their phosphatase activity. We found similarly that the rhinoceros's milk with its high content of phosphorylated thiamine was very low in alkaline-phosphomonoesterase activity.

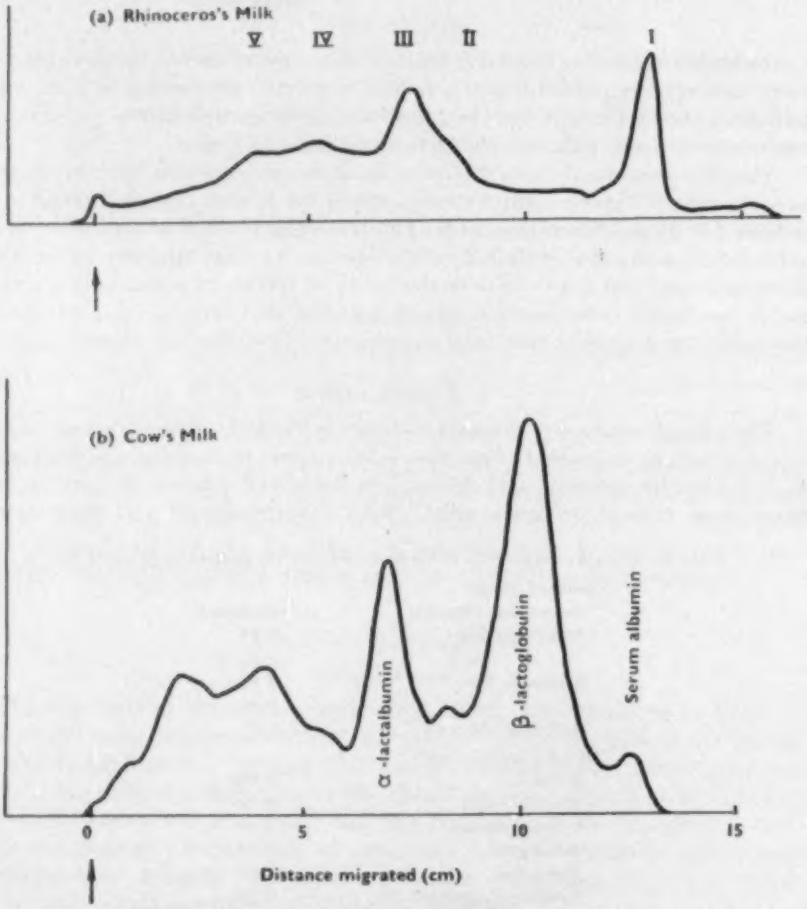

Fig. 1—Electrophoretic patterns of the soluble proteins of (*a*) rhinoceros's milk and (*b*) cow's milk.

Values for riboflavin obtained fluorimetrically were three times higher than those obtained microbiologically. We are at present unable to account for this discrepancy. The most probable explanation is that the milk contains substances that behave like riboflavin in the fluorimetric tests, but are biologically inactive. Such substances were shown by Pearson & Schweigert (1947) to occur in the milk of goats given large quantities of riboflavin.

SUMMARY

1. A sample of milk of the African black rhinoceros (*Diceros bicornis* ; Linn) taken during the mid to late lactation period was analysed for fat, solids-not-fat, lactose, protein, casein, soluble proteins, non-protein nitrogen, ash, calcium, phosphorus, sodium, potassium chloride and iron. The milk contained only a trace of fat and less protein and calcium than cow's milk but more lactose.

2. Paper electrophoresis resolved the soluble protein fraction into at least five components.

3. The total ascorbic acid, calcium pantothenate and vitamin B_{12} contents were similar to those in cow's milk, whereas values for nicotinic acid, biotin, riboflavin and vitamin B_6 were lower and values for thiamine higher.

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